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Gestation and early-life environment and its impact on welfare and cognition in pigs

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Abstract

Assessing cognitive capabilities of animals is key to understanding their welfare needs. This is especially the case during major life events or following a change in conditions, when cognition and welfare needs may change or fluctuate. One such major life event is gestation, when a female's physical and physiological environment can change significantly. In livestock, pregnant females are also frequently subjected to an environmental change, as they are typically separated from other conspecifics. The aim of this thesis was to investigate the impact that gestation and early life environment may have in pigs. This thesis includes two main studies, the first of which focuses on the impact of pregnancy on cognition and mood, investigating memory, problem solving and personality throughout pregnancy. This is also the first study, to my knowledge, to use a cognitive bias approach to assess changes in mood during pregnancy in a non-human animal. In doing this it was found that there is a significant shift in the pigs' mood state between pre/early and mid/late gestational stages, suggesting that they became increasingly pessimistic as they progressed through pregnancy. The second study investigates how two key early life factors, the size and sex ratio of the litter an individual is born into, may influence its later body and tail injury scores as well as cognitive bias when the pigs are placed in mixed groups in either a barren or enriched environment. A key finding from this study is that the sex ratio of the litter an individual originates from can have an effect on its body scores after leaving its litter-mates. It was also found that litter size and relative weight of the individual within the group can impact upon tail injury scores, though the nature of the effect also depends on the pigs' housing environment. Results correlate with previous findings that litter size is associated with litter sex ratio. Overall this thesis provides an initial insight into the impacts of pregnancy on a mother's cognitive bias and suggests that environmental factors in utero and in early life can influence welfare in later life.

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Chapter 1 - Introduction

Mammalian pregnancies vary considerably between species; for example, the human gestation period is approximately 40 weeks in duration, though other species have gestation periods very much shorter than this, such as the opossum who has a gestational length of approximately 13.5 days (Mate, *et al*., 1994), and others are considerably longer, such as 22 months in African elephants (Allen, 2006), with many other species falling between these limits (e.g. African Buffalo, approximately 11 months: Ryan, et al., 2007; Sun Bear, approximately 3-3.5 months: Schwarzenberger, *et al.,* 2004; rhesus macaques, approximately 166.5 days: Silk, *et al.,* 1993).

In addition to variations in gestation period, the placenta can vary in structure and physiology between mammalian species (Abbot & Rokas, 2017; Leiser & Kaufmann, 1994), and although the main roles of specific hormones, such as oestrogen and progesterone may remain the same, endocrinological patterns vary across species (Humans: Soldin, *et al*., 2005; Llama: Leon, *et al*., 1990; Rat: Purl & Garfield, 1982; Pig: Robertson & King, 1974). Despite differences, it is apparent that pregnancy affects the individual's physical and physiological state in all mammals.

Cognition may be defined as the process or mental action required in order to gain knowledge and understanding via experience, thought and the senses (Griffin, *et al*., 2015; Duncan, & Barrett, 2007). It encompasses many functions within the brain including attention, memory, social learning, associative learning, judgement and reasoning to name a few (Griffin, *et al*., 2015). Gestation has been associated with changes in cognitive function and mood (Table 1) (Galea*, et al*., 2000; Buckwalter, *et al*., 1998). For example, changes in oestrogen and progesterone levels have been shown to impact on learning and spatial memory (Kinsley, et al., 2006), key capabilities for successful rearing and survival

in many species (Kinsley, *et al*., 1999). In rats, studies have found that oestrogen and progesterone induce modifications in the hippocampus, enhancing navigation and spatial abilities required for foraging-type behaviours during gestation and early motherhood (Darnaudéry, *et al*., 2007; Kinsley, et al., 2006; Kinsley, *et al*., 1999). By contrast, research in humans has frequently concluded that pregnancy has a significant negative impact on spatial memory (Farrar, *et al*., 2014; Anderson & Rutherford, 2012; de Groot, *et al*., 2006), though there is lack of consensus on the conclusion that pregnancy effects overall memory, as other researchers have found no effect at all (Logan, *et al*., 2014; Christensen, *et al*., 2010). This lack of consensus may be due to the variety of different tests being used to assess many different components of memory, including implicit, explicit, spatial and working memory (Burgess, *et al*., 2002; Ericsson & Kintsch, 1995; Gabrieli, *et al.,* 1995). For example, Glynn (2010) used a face recognition paradigm, which required each participant to remember 24 faces, and found no recognition memory deficit during pregnancy, whereas Farrar, et al. (2014) found that spatial recognition memory significantly declined in pregnant women where the participant had to identify the correct original location of an object. It is clear that the variety of tests used, assessing different components of cognition, makes it difficult to draw conclusions on the effect of pregnancy on memory. Overall, research into the effects of pregnancy on maternal cognitive function is inconclusive, and has been conducted in just two species (humans and rats). The observed differences in performance between the species may, in part, be linked to differences in brain structure which are likely to impact cognitive processing (Pawluski, *et al*., 2016; Duarte-Guterman, *et al*., 2015; Gieling, *et al*., 2011b). There is some suggestive evidence in humans that the nature and magnitude of alteration in maternal cognition is associated with the sex of the foetus (Al-Qaraghouli & Fang, 2017; Vanston & Watson, 2005; del Mar Melero-Montes & Jick, 2001). For example, one study found that women pregnant with a single male foetus consistently outperformed women carrying a single female foetus in tests of working memory and spatial ability (Vanston & Watson, 2005). This suggests there is a sex-specific effect of the foetus on the maternal

brain and this may potentially account for the mixed findings in relation to cognitive function during pregnancy (Lombardo, *et al*., 2012; Bos, *et al*., 2010). These kinds of studies investigating the effects of foetal sex on maternal cognition are intriguing, although often the possible effect of foetal sex is neglected in pregnancy studies.

Table 1: A non-exhaustive overview the literature focusing on mood and cognition during pregnancy.

Aspect of cognition	Species	Stage of Pregnancy	Findings	Reference
Facial recognition	Humans	Not specified	Pregnant women were better at recognition of faces.	Anderson & Rutherford, (2011)
Mixed	Humans	Third trimester and postpartum	Some but not all aspects of cognition were impaired during pregnancy	Buckwalkter, *et al*. (1999)
Mental speed, working memory and immediate & delayed recall	Humans	Early and late pregnancy	No effect of pregnancy on cognitive function	Christensen, *et al*. (2010)
Spatial memory	Rats	14 days gestation	No difference in spatial memory during pregnancy	Cost, *et al*. (2014)
Verbal memory, divided attention, & focused attention	Humans	Second & third trimester & postpartum	Women showed a perceived cognitive impairment but no actual impairment was found	Crawley, *et al*. (2003)
Mental speed & memory	Humans	Throughout pregnancy and postpartum	Memory, but not mental speed, was impaired during pregnancy	De Groot, *et al*. (2006)
Spatial working memory, attention recognition memory &	Humans	Throughout pregnancy	Reduced spatial memory performance	Farrar, *et al*. (2014)
Depression	Humans	Postpartum	Women who experience depression during pregnancy are more likely to suffer from postpartum depression	Gaillard, *et al*., (2014)
Spatial working memory	Rats	Throughout pregnancy	Spatial working memory was improved during first and second trimesters but was worse than non-pregnant rats	Galea, *et al*. (2000)

			during the third trimester	
Working, recognition & verbal recall memory	Human	Throughout pregnancy	Verbal recall memory performance declined with advancing gestation. No difference in working or recognition memory	Glynn, (2010)
Working memory	Humans	Third trimester of pregnancy	Overall no difference in working memory between pregnant women and controls. Only women experiencing depression had impaired working memory.	Hampson, *et al.* (2015)
Explicit memory	Human	Throughout pregnancy and postpartum	Decline in memory during the third trimester only.	Keenan, *et al*. (1998)
Learning and memory	Rats	Throughout pregnancy	Learning and memory improved	Kinsley, *et al*. (1999)
Memory and attention	Human	Throughout pregnancy and postpartum	No cognitive deficit was found but women reported self-perceived memory decline throughout pregnancy	Logan, *et al*. (2014)
Depression	Humans	Throughout pregnancy	20% of the 3472 women tested were experiencing symptoms of depression	Marcus, *et al.,* (2004)
Working & reference memory & learning	Rats	Postpartum	Improved learning and memory	Pawluski, *et al.* (2006)
Depression	Humans	Mid-pregnancy and 6 months postpartum	Of 1662 women, 9% experienced depression mid-pregnancy and 8% postpartum	Rich-Edwards, *et al.* (2006)
Anxiety & depression	Humans	Throughout pregnancy	Anxiety was higher during the first and third trimesters. Muliparous women had higher depression scores in the third trimester while Primiparous women had higher depression scores during the first trimester.	Teixeira, *et al*. (2009)
Anxiety & depression	Humans	Throughout pregnancy	No significant difference between the prevalence rate of anxiety and depression between pregnancy and non-pregnant women.	Ugaz, *et al*. (2010)

Studies investigating prenatal stress in humans have found that maternal stress and anxiety can negatively impact on the child's mental wellbeing, resulting in a greater risk of mental health problems in the future. A study by Buss, *et al*. (2010) found that high levels of anxiety during pregnancy, or more specifically at the 19 weeks stage in human mothers, can significantly reduce grey matter volume in the foetal brain. These changes specifically occurred in areas associated with cognitive performance and emotion, such as the prefrontal cortex (Sullivan, 2004). This has the potential to impact cognitive development, as shown by Brouwers, *et al*. (2001) who found that infants by the age of two have lower mental development scores on average if their mother experienced anxiety throughout her pregnancy. Not only this but prenatal stress and anxiety can alter the offspring's HPA axis function (Frodl & O'Keane, 2013; Glover, *et al*., 2010; Van den Bergh, *et al*., 2008; Bennet & Gunn, 2007). The HPA axis is the main neuroendocrine stress response system in the body, responsible for control of production of the main stress hormone, cortisol, from the adrenal glands (Frodl & O'Keane, 2013). Alterations to the HPA system experienced *in utero* have the potential to effect long term responses to stress, as shown by Clarke, et al. (1994) who found that offspring from stressed rhesus monkeys have an enhanced more sensitive HPA axis response to stressors in adulthood, and the same outcome has been found in rats (Darnaudéry & Maccari, 2008; Henry, *et al*., 1994). Similar research by Coe, *et al.* (2003) found that pregnant rhesus monkeys subjected to unpredictable noise during early or late pregnancy produced offspring with smaller hippocampi; furthermore Szuran, *et al*. (1994) found that prenatally stressed rats had a reduced hippocampal weight. The hippocampus is a brain structure vital for the control of the HPA axis and memory (Frodl & O'Keane, 2013; Jacobson & Sapolsky, 1991) and a few studies have specifically linked prenatal stress with altered synaptic plasticity in the hippocampus, consequently impacting upon learning and memory (Lui, *et al.,* 2011; Yang, *et al*., 2007; Yang, *et al*., 2006). Whilst this prenatal programming could be beneficial for wild populations, helping to promote resilience and survival (Anderson & Rutherford,

2012; Love & Williams, 2008) in domesticated or captive populations, this may be unnecessary and potentially have adverse consequences.

A relatively small number of studies have reported sex differences in foetal programing, often showing that males are more affected by adverse *in utero* conditions than females (Aiken & Ozanne, 2013; Sandman, *et al*., 2013; Baxter, *et al.,* 2012; Eriksson, *et al*., 2010). For example, human male foetuses are more likely to be spontaneously aborted or miscarried (Vatten & Skjærven, 2004). Other studies have shown that sex differences are linked to specific periods within the gestation period. Mueller & Bale (2008) showed that male mice that experience prenatal stress early in gestation were significantly more immobile when taking part in a forced swim and tail suspension test. This result, characteristic of a depressive phenotype, was not seen in female mice that were exposed to early prenatal stress. Another study showed that in rats the dentate gyrus was affected differently in male and female offspring exposed to prenatal stress. Male offspring had higher dendritic spine complexity and density in this area, while females experienced dendritic atrophy (Bock, *et al*., 2011). These are just two findings from two studies among others (Bock, *et al*., 2015; Behan, *et al*., 2011; Zuena, *et al*., 2008) that highlight how males and females can respond differently to conditions in-utero.

Stress does not only impact upon the foetus but also the mother, as prenatal stress can increase the risk of post-natal depression in human mothers (Leung, *et al*., 2005). Mood and mental health issues are common among pregnant women (Table 1) and often attributed to the extreme variations in hormones during this period of time (Workman, *et al*., 2011; Bennet, *et al*., 2004). Levels of maternal care have been shown to be related to maternal mood (Lovejoy, *et al*., 2000). In both human and non-human mammals, oxytocin and dopamine pathways are key mediators of maternal care (Kim, *et al*., 2016). Increasing immediately after birth and during sucking (Nissen, *et al.,* 1995), oxytocin promotes bonding between mother and infant (Douglas, 2010) and facilitates maternal behaviour in

human and non-human mammals, such as nest building, licking, grooming and nursing (Ross & Young, 2009; Kendrick, 1987). In humans, dopamine-associated rewarding areas of the brain are activated when a mother views their child's face as happy (Strathearn, *et al*., 2008), again reinforcing the care of the infant. This reward circuit is also linked to the amygdala, which is activated in response to infant cues or stimuli, such as smiling and crying (Ross & Young, 2009; Strathearn, *et al*., 2008). This activation is generally interpreted as a positive response in human mothers, however in rats it has been associated with a reduction in maternal behaviour (Riem, *et al*., 2012; Ross & Young, 2009), again highlighting how varied results can be between species.

A larger amount of research in recent years has focused on the experiences and environment *in utero* and the perinatal period and how this may influence offspring development. Typically termed foetal or prenatal programming, this has been investigated in a number of mammals, including humans (Godfrey & Barker, 2000), rats (Darnaudéry & Maccari, 2008), pigs (Foxcroft, *et al*., 2006; Jarvis, *et al*., 2006) and chimpanzees (Murray, *et al.,* 2016), focusing on those factors considered to be most influential, such as maternal diet, mental health and infection (Bale, *et al*., 2010; Charil, *et al*., 2010; Heerwagen, et al., 2010). There are large differences in degree of maturity and independence between mammalian offspring. For example, human infants are born fully dependent (Rosenberg & Trevathan, 2002), whereas marsupials are born in embryonic state although they can climb unaided to its mother's teat (Keyte & Smith, 2010), and other mammals give birth to highly developed precocial offspring that can walk within hours of being born, such as calves and foals (Gorissen, *et al*., 2016). Differences in the physical maturity and independence of different mammalian species' offspring can impact on the duration and nature of the perinatal period. In humans the World Health Organisation defines the perinatal period from 22 weeks of gestation to one week after birth (WHO, 2017), however the length of this period is relative to the species and length of gestation. For example, the perinatal period for mice would be only a few days either

side of birth (González-Menéndez, *et al*., 2011), where as for cats and dogs one paper by Lodge, *et al.* (2012) describes the perinatal period as "20 weeks prior to birth until 4 weeks after". Maternal care during the perinatal period also has the potential to affect the offspring's cognition (Tamaroff, *et al*., 1986), with research in rats showing a direct link between maternal care received and development of the hippocampus (Liu, *et al*., 2000). Rats that received more licking and grooming from their mother as pups are less fearful as adults and females also tend to have a higher number of oxytocin receptors in the brain, suggesting that they are likely to be effective mothers themselves later in life (Francis, *et al*., 2002). Absence of maternal care during the perinatal phase can be harmful, with some studies in rats showing how maternal separation may cause cognitive impairment that lasts throughout life (Sousa, *et al*., 2014; Aisa, *et al*., 2007). Similarly to humans, rats that experienced stress as a juvenile are more likely to exhibit anxiety related behaviours as an adult and are also more optimistic and quicker to make decisions (Brydges, *et al*., 2012). This impulsivity has also been seen in human infants whose mothers had a high level of prenatal anxiety (Van den Bergh, *et al*., 2005).

Whilst much has been studied in this area, it has been done so primarily in either humans or rats, and as such there is much less known about how gestation and the early life period impact on other species. Previous research in other species, such as livestock, has also shown that the gestational and early-life period are influential for mother and offspring (Rooke, *et al*., 2017; Rooke, *et al*., 2015;), for example Rutherford, *et al*. (2014) demonstrated that prenatal stress not only effected piglet survival but also on sow maternal behaviour.

Domestic pigs are a good choice of model for two reasons: firstly, humans keep and breed millions of them annually with 4,815,00 pigs in the UK, and 985,673,301 worldwide in 2014 (Food and agriculture organisation for the United Nations, 2017) and a better understanding of this period is needed in order to refine husbandry conditions for

improved welfare. Secondly, pigs have been used as a model for humans in medical research, so this would make it an interesting comparison to the laboratory rat model. Commercially farmed pigs experience multiple challenges and stressors throughout their lifetime, including weaning, mixing and pregnancy. This thesis focuses on two significant times in the life of a commercial pig: pregnancy and early life. A large focus of previous research in this area is the welfare issues surround farrowing crates as although they are designed to prevent the crushing of piglets they confine the sow, restricting movement and therefore the expression of maternal nesting behaviours often resulting in stress (Wischner, *et al*., 2009; Jarvis, *et al.,* 2002; Thodberg, *et al.,* 2002). There are many studies on behavioural needs of gilts and sows during pregnancy, such as nest-building behaviour, and its impact on overall welfare (Algers & Uvnäs-Moberg, 2007; Boyle, *et al*., 2002; Damm, *et al*., 2003; Wischner, *et al*., 2009), or comparing farrowing crates to alternatives that allow varying levels of space, including farrowing pens (Damm, *et al*., 2003) and family systems (Arey & Sancha, 1996). There have also been some studies showing the negative effects of large litter sizes which can often lead to inter-uterine crowding, resulting in compromised performance, viability, thermoregulatory abilities, birth weight and an increased risk of mortality shortly after birth (Bérard *et al*., 2010; Quiniou, *et al*., 2002; Wilsson & Sundgren, 1998; Herpin, *et al*., 2002; Andersen, *et al*., 2000; Auldist, *et al.*, 1998). Research has investigated the effect of prenatal stress on piglets, often focusing on its impact upon the immune system (Couret, *et al*., 2009a; Couret, *et al*., 2009b; Tuchscherer, *et al*., 2002). Although here has been some research investigating how physiological changes during pregnancy can affect offspring in pigs there has been little research investigating how pregnancy may impact on the cognitive function of the sow or gilt.

Pigs as models for both their own and human cognition have many advantages. They are in many ways anatomically and physiologically similar to humans (Merrifield, *et al*., 2011; Sullivan, *et al*., 2001) and their brain closely resembles the human brain, both in

biochemistry and structure (Kornum & Knudsen, 2011). Typically rodents are used in studies investigating the effect of pregnancy on cognition, however rodent brains have large structural differences, such as their lissencephalic brain in comparison to the human and porcine brain, which is gyrencephalic (Lind, *et al*., 2007). The cerebral cortex is an area of the brain responcible for complex behaviours, cognitive function and sensory abilities (Sun & Hevner, 2014) and so when using choosing a species as a model for another it is preferable to use one that has as a similar brain structure. Brain development also occurs at different times. The rodent brains developing postnatally, where as the porcine brain, like the human brain, develops perinatally with the main growth spurt occurring from mid-gestation through to the early postnatal period (Jelsing, *et al*., 2006; Pond, *et al*., 2000).

Understanding the cognitive capacity of animals and how cognitive function can change in relation to changes in environment, physiology and other challenges throughout life is key to understanding its welfare needs. Increasing and optimising the welfare of farm animals is not just beneficial to the animal but can have benefits for production by reducing adverse health conditions, deaths and increasing efficiency (Held, *et al*., 2002; Curtis & Stricklin, 1991). Pigs have the ability to learn operant and classical conditioning tasks quickly (Gieling, *et al*., 2011a), something that is an advantageous when carrying out cognition research. In light of this, pigs cannot only assist understanding of human cognition but also their own cognition and aid understanding of how their welfare can be improved in relation to current intensive farm rearing systems.

1.1 Thesis aims

This thesis had two main aims:

(i) To investigate the impact of pregnancy on cognitive processes and mood in primiparous gilts

(ii) To investigate effects of prenatal and early life factors, including litter size and sex ratio, on cognition and health in pigs.

Chapter two focuses on maternal cognition during pregnancy and investigate changes in memory, problem solving and cognitive bias across the gestation period. Studies exploring memory during pregnancy typically use humans or rats as a study species, therefore this thesis also aimed to bridge gaps in the literature by using a non-typical study species for this area of research. A cognitive bias approach is used to investigate the impact of pregnancy upon mood/mental state and this is the first study, to my knowledge, to look into this in a non-human animal.

Chapter three presents novel findings from analysis of a large dataset collected for a previous BBSRC-funded study, investigating how sex ratio and size of an individual's birth litter may influence how they cope in different environments in later life. As part of this, body and tail injury scores are analysed in relation to sex ratio and litter size. There are also novel analyses of data from a cognitive bias test conducted on a sub-sample of these pigs, in order to test the hypothesis that judgement bias is linked to prenatal and early life factors.

Chapter 2 - Investigating the impact of pregnancy on mood and cognitive processes in a non-human animal

Abstract

Pregnancy causes multiple different changes in hormones, mood and cognitive ability in human and non-human mammals. There has been some research into how these adjustments affect cognition and mood in humans, however non-human literature is focused on rodents. The aim of this study was to investigate the impact of pregnancy on cognitive processes in a non-human animal, the domestic pig (*Sus scrofa domestica*), which often experiences consecutive pregnancies throughout their lifetime. This is the first study, to my knowledge, to use a cognitive bias approach to investigate mood/mental state during pregnancy in a non-human animal. Another aim was to investigate the potential mediating/moderating effects of personality on the cognitive impacts of pregnancy on an individual. After training, 10 gilts were tested at four different stages of the 16-week gestational period; before, early (5 weeks), middle (8 weeks), and late (11 weeks) pregnancy. Testing included cognitive bias tests at each stage. A five trial 'reminder' session of alternating positive and negative reference probes (P, N, P, N, P) was conducted on the day before each test day; this 'reminder' session was also used to assess memory. Individuals also completed three further tests: puzzle solving, novel object and social isolation tests at the before, early and late test stages of pregnancy in order to assess problem-solving ability and personality. There was a significant increase in latency to reach the cognitive bias test probes between before/early and middle/late stages of pregnancy, suggesting a significant shift in the pig's mood state, as they become increasingly pessimistic towards the latter stages of pregnancy. There was also a significant effect of personality on the pig's responses to the cognitive bias test, with proactive individuals having a more pronounce difference between latencies to reach the

ambiguous locations at the before/early and middle/late test times. The gilts did become significantly faster to solve the puzzles in the problem solving test as their pregnancies progressed, however no significant change in memory was found when comparing before, early, middle and late stages of pregnancy. Overall this study begins to show how pregnancy may influence mood and cognition in a non-human animal and provides a basis for future work in this area.

2.1 Introduction

In the human-based literature, the state of gestation (pregnancy) has been associated with changes in mood, mental state and cognitive ability (Macbeth & Luine, 2010; Henry & Rendell, 2007; Bennett, *et al*., 2004). Mood disturbances, including depression and anxiety, are common during pregnancy amongst women with prevalence dependent on ethnicity, parity and socioeconomic factors (Lancaster, *et al*., 2010; Teixeira, *et al*., 2009; Rich-Edwards, *et al*., 2006; Marcus, *et al*., 2003). Some studies have linked these emotional and mental states to the hormone fluctuations that occur rapidly during the gestational period (Workman, *et al*., 2011; Uguz, *et al.,* 2010; Buckwalter, *et al*., 1998). In particular, the fluctuations in peptide and steroid hormones, such as oestrogen and progesterone (Steiner, *et al.*, 2003), which can also greatly affect the maternal brain (Kinsley, *et al*., 2006; Oatridge, *et al*., 2002). A recent study comparing brain structure before and after pregnancy in first time mothers found substantial differences in grey matter, with first time mothers undergoing a significant reduction in grey matter throughout pregnancy (Hoekzema, *et al*., 2017; Oatridge, *et al*., 2002). Other research by Farrar, *et al*. (2014) found that pregnancy negatively affected spatial recognition memory performance, specifically during the second and third trimester in pregnant women, a result that supported earlier studies (de Groot, *et al*., 2006; Keenan, *et al*., 1998). A review by Anderson & Rutherford (2012), aimed to update a previous meta-analysis by Henry & Rendell (2007), also found a small but significant negative impact of pregnancy on

working memory. Despite this, many studies have found no cognitive deficit during pregnancy (Logan, *et al*., 2014; Christensen, *et al*., 2010; Crawley, *et al*., 2003), or have suggested that many self-reported perceived cognitive deficiencies are related to mood rather than any actual cognitive deficit (Logan, *et al*., 2014; Marino, *et al*., 2009).

Non-human literature investigating links between memory and pregnancy has generally reported research on rats, which have large differences in brain structure and hormonal patterns during gestation in comparison to humans (Pawluski, *et al*., 2016; Duarte-Guterman, *et al*., 2015; Gieling, *et al*., 2011b). In rats it is often found that there is a decreased rate of neurogenesis in the hippocampus, an area of the brain directly sensitive to hormonal changes and critical for the regulation of mood, memory and spatial ability (Galea, *et al*., 2013; Workman, *et al*., 2011; Bunsey & Eichenbaum, 1996), although spatial memory is enhanced (Cost, *et al*., 2014; Macbeth, *et al*., 2010; Galea, *et al*., 2008; Galea *et al.,* 2000). Indeed much of the research published in this area of the non-human literature focuses on spatial memory (Cost, *et al*., 2014; Macbeth, *et al*., 2010; Galea, *et al*., 2008; Galea *et al.,* 2000), whereas studies looking at, other types of memory during pregnancy are more limited in number, and typically focus on reproductive experience. For example, Pawluski, *et al*., (2006) who found that rats that were first or second time mothers had enhanced working and reference memory. Conflicting results across the human and non-human animal literature means that understanding of the impacts of the gestational period and process on memory is very limited, and very little is known about how gestation may effect memory or other cognitive processes in species other than humans and rodents.

A growing body of research in both human and non-human animals suggests that cognitive processes are not just influenced by hormonal changes, such as those during gestation, but also by mood and emotions, causing attention, judgment and memory to be altered in the short, or even long term (Boissy, *et al*., 2007). Cognitive bias, or judgement

bias, is the influence of affect, mood state and emotion on cognition, with happy, content individuals more likely to make positive assumptions about ambiguous stimuli (Bethall, *et al*., 2015). In humans, a negative mood state has been shown to lead to a pessimistic cognitive bias (Sharot, 2011; Mathews & MacLeod, 2005). Sharot, (2011) explains that people tend to overestimate the likelihood of future positive experiences happening in comparison to negative outcomes, even if the chance of future positive or negative experiences is equal. Increased pessimism has been reported in individuals experiencing depressive symptoms, with severely depressed individuals having a significant pessimistic bias (Strunk, *et al*., 2006; Lothmann, *et al*., 2010). Non-human animals cannot convey their perception of their mental state as easily as humans can, and as such their affective state must be assessed indirectly (Bateson, *et al*., 2011). There have been a number of studies published in recent years that have focused on using cognitive bias in a wide range of species including dogs, pigs, rats, European starlings and even bees (e.g. pigs: Asher *et al*., 2016; pigs: Douglas, *et al*., 2012; bees: Bateson, *et al*., 2011; rats: Brydges, *et al*., 2011; dogs: Mendl, et al., 2010; European starlings: Bateson & Matheson, 2007). For example, in pigs, cognitive bias studies include that of Douglas, *et al*., (2012) who found pigs housed in enriched environments respond more 'optimistically' in testing, and Scollo, *et al.,* (2014) found that pigs living in pens with different stocking density levels did not show different judgement bias. Although there are a large number of studies that use the cognitive bias test, they have typically been used to assess the impact of externally driven factors on animal cognition and welfare, rather than internally driven factors (such as pregnancy).

Although not included in cognitive bias studies until recently, personality has been shown to impact individuals' cognitive bias in a context-dependent manner (pigs: Asher, *et al*., 2016). Animal personalities, also referred to as "behavioural syndromes", are defined by a set of consistent individual differences in behaviour across time and context (Sih, *et al*., 2004), and can be divided into two main types; proactive and reactive. More reactive

personality types are associated with more flexible and passive behaviour, whereas proactivity on the opposite end of the scale is associated with more active and less flexible behavioural responses (Koolhaas, *et al*., 1999). Including this factor in future studies may account for variation in cognitive bias results within and between studies (Asher, *et al*., 2016).

The gestation period for commercial pigs will typically present a number of internally driven and external factors that can impact on the female's welfare and cognition. A large amount of research investigating pregnancy in pigs focuses on their environment as during gestation sows are typically moved into a farrowing crate, designed to reduce piglet mortality by crushing, but also allow for easier access for the stock person to assist with delivery. Farrowing crates are restrictive for the sow, with only space to stand up and sit or lie-down, restricting the expression of other behaviours, including maternal behaviours, such as nest building (Algers & Uvnäs-Moberg, 2007; Grandinson, 2005; Blackshaw, *et al*., 1994). Maternal behaviours are induced by changes in specific hormones such as prolactin and progesterone (Algers & Uvnäs-Moberg, 2007). Various studies have been conducted that suggest that the type of environment the female is housed in during pregnancy impacts on maternal behaviour. Arey, *et al*. (1991) who found that sows presented with straw, sand and enough space would build nests and display other maternal behaviours such as pawing and rooting. Jarvis, *et al.* (2002) also explored whether material availability or space had a greater impact on sows, finding that individuals housed in a pen rather than a crate showing more locomotive behaviour, such as standing, walking and changing position. Although pens and other open farrowing systems may increase sow welfare, it often increases the risk of piglet mortality by crushing as shown by many studies (Weber, *et al.,* 2007; Weary, *et al*., 1996) including Blackshaw, *et al*. (1994) who found that in a farrowing crate there was 14% piglet mortality in comparison to 32% in the farrowing pen. However, despite the increased risk of mortality, open pen systems also

allow for the piglet to display play behaviours, which is thought to both increase welfare and cognitive development (Martin, *et al*., 2015).

Previous research investigating the possible links between pregnancy, cognition and mood has had variable conclusions (Anderson & Rutherford, 2012) and is mostly carried out from a human perspective, or has involved rodents, which have large differences in brain structure in comparison to humans (Gieling, *et al*., 2011b). Pregnancy involves cognitive, emotional and hormonal changes, which all have the potential to influence each other and change throughout different stages of gestation (Soldin, *et al*., 2005; Steiner, *et al.*, 2003). Therefore, the aim of this study was to investigate the impact of pregnancy on cognitive processes, including mood, problem solving and memory in a non-human animal by focusing on four main hypotheses:

(i) Pregnancy will influence cognitive bias and cognitive bias will vary depending on the stage of pregnancy.

(ii) Problem solving ability will change during pregnancy in comparison to before pregnancy.

(iii) Memory will be altered during all stages of pregnancy relative to memory before pregnancy.

(iv) Personality will explain some of the variation in cognitive bias at different stages of pregnancy.

2.2 Method

2.2.1 Animals and housing

Ten gilts; six Large White, three Landrace and one Duroc, were selected based on age and time until first service. The average age of all ten pigs on the first day of training was 7.1 months with a standard error of 0.18. Gilts were selected as they were all due to

experience first parity, which meant that prior pregnancy experience was the same for all individuals. This also allowed more time for training and testing than would be possible if higher parity sows were recruited, due to the short length of time between consecutive pregnancies in commercially farmed breeding sows.

The ten gilts were situated on a high health breeding farm and were housed as two groups of six, group A and group B, including one gilt per pen that was not used in the experiment. The gilts were grouped according to the farmer's preference while ensuring there were equal numbers of test individuals in each pen. Group A consisted of six Large Whites, (one gilt was not included in the study due to a foot infection), and group B consisted of one Large White, three Landrace, one Duroc and one Welsh. The Welsh gilt had a nervous disposition and so on ethical grounds, it was decided not to include it in the study in case the training and testing negatively impacted its welfare. Pens were located in the same building, each pen measured 4.67m x 5.35m and contained a sheltered sleeping area (2.70 x 4.67m) and a run partially exposed to outdoor elements, such as natural light and wind, (2.65 x 4.67m) to which they had continuous access. Each pen had the same level of enrichment, consisting of a covering of straw on the ground and a larger amount in the sheltered sleeping area. All individuals were fed a standard lactating sow ration once a day, had continuous access to water and natural lighting.

2.2.2 Personality testing

Personality testing was carried out in the same was as detailed by Friel, et al. (2016) due to its previous success in pigs. Testing was carried out at the before, early and late pregnancy stages and consisted of a social isolation test and a novel object test as previously described by Friel, *et al*. (2016). The training and testing area used comprised of a testing room (3.72 x 5.26m), a starting room (3.72 x 1.79m) and a holding area (3.00 x 3.30m). All individuals had been previously habituated to the test room as part of training for the cognitive bias test. Social isolation tests were conducted the day before the novel object

test and involved each individual being placed into the test area for three minutes on their own. The novel object test lasted five minutes and involved each individual entering the test room containing an object they had never seen before (figure 1). A traffic cone, grey box, stool and blue bucket were all used as novel objects as they all differed in shape size and colour. Each pig received a new object each time they completed the test and objects were counterbalanced between pig and test time to avoid any bias. Each test was repeated twice per pig before pregnancy, then repeated on two further occasions, once at the early pregnancy stage and once at the late pregnancy stage. The time spent moving, standing, drinking and exploring the floors and walls were measured from the footage of the social isolation and novel object tests. The time to approach the novel object and the time spent interacting with the object were also measured for the novel object test.

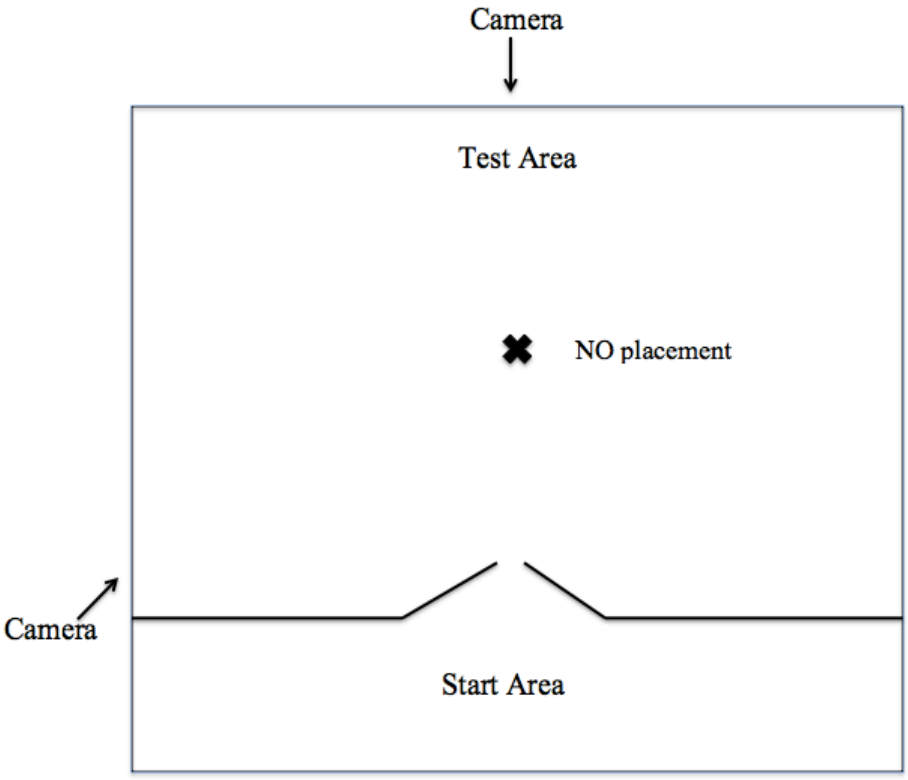


Figure 1: Experimental set up for the novel object test, where the novel object would be placed in the middle of the testing area. This same set up but without any objects in the room was used for the social isolation test.

2.2.3 Cognitive bias

A spatial cognitive bias design was decided upon based on previous success with pigs using this type of design (Asher, *et al*., 2016).

The training and testing area used comprised of a testing room (3.72 x 5.26m), a starting room (3.72 x 1.79m) and a holding area (3.00 x 3.30m). Before training, each pig was individually marked using a non-toxic spray marker applied to the back.

The gilts were trained to discriminate between positive (P) and negative (N) locations based on their outcome, i.e. to approach the P location for a reward (Sugar coated chocolates) and to avoid the N location as it was unrewarded. To assess the pigs responses and cognitive bias, latency to reach the bowl was used. Each trial was 30 seconds long and during the positive trials the nose touching the bowl was marked as a correct response as was not touching and avoiding the bowl during the negative trials. Within each group, the pigs positional cues were counterbalanced so two of five in one pen were trained with the P location in the right-hand corner and three were trained with P location in the left-hand corner, and in pen two, three were trained with P location in the left-hand corner and two were trained with P location in the right-hand corner.

Training was carried out individually for 15 sessions, with an average of six trials per session, totalling 97 training trials per individual. For the first 36 trials the negative (N) bowl was empty, this was because in a previous pilot study we found that some pigs would attempt to eat any aversive food/substance in the negative location. Previous studies in dogs (Mendl, *et al*., 2010) and pigs (Scollo, *et al*., 2014) have left the negative location empty and so this method was adopted. However, the pigs were not learning to discriminate between the positive and negative fast enough or at all and so the decision was made to add coffee beans to the negative location, which pigs find aversive. This method has worked in previous studies with pigs (Asher, *et al*., 2016). From trial 37-97 the

N bowl contained coffee beans, Bowls were secured to the floor to prevent movement. The positive (P) bowl contained chocolate as a reward throughout all P training trials. Typically there is a criterion that needs to be met before moving onto the testing phase (e.g. 75% correct responses in the last 20 trials), due to time constraints and the issue with negative reinforcement, there was no criterion for the transition from training to testing in this study.

Cognitive bias testing was done on four separate occasions; before mating, then five, eight and eleven weeks post-mating. As the gestational period is 115 days (approximately 16 weeks), this allowed for assessment of before, early, middle and late pregnancy. After the first test time 'before', the gilts were served over a period of three weeks due to their varying oestrous cycles. The following test time were done at the appropriate time for each individual. A group of pigs being tested on one day were not always tested in the same order. Testing involved five different probe/bowl locations; a positive (P) and a negative (N) reference location in each corner and three ambiguous probes; near positive (NP), middle (M) and near negative (NN), all equidistant from each other (0.74m). Only one bowl was in the area per test. Tests were unreinforced; all ambiguous bowl locations were empty, though P and N bowl locations were reinforced in between the ambiguous testing probes to maintain motivation to respond and guard against extinction over the test session. The positive, negative and ambiguous bowls were never mixed and were rinsed with water between each trial.

Each of the four testing sessions comprised 18 trials carried out on the same day, with each individual receiving a different sequence order at each different test time. The trial sequences involved two of each of the ambiguous probes (NN, NP, M), resulting in six ambiguous locations per test. These were interspersed with positive (chocolate) and negative (coffee) trials, for example; P, N, *NN*, P, N, *M*, N, P, *NP,* to make up the 18 trail sequence and to prevent the pigs becoming disinterested. All 'during pregnancy' testing

sessions were preceded by a five trial 'reminder' session of alternating reference probes (P, N, P, N, P) the day before, ensuring all individuals were familiar with the exercise.

Unfortunately only nine pigs were tested 'before' pregnancy as farm staff served one pig early. All test sessions including personality and problem-solving tests were recorded using a Sony CX625 Handycam video camera.

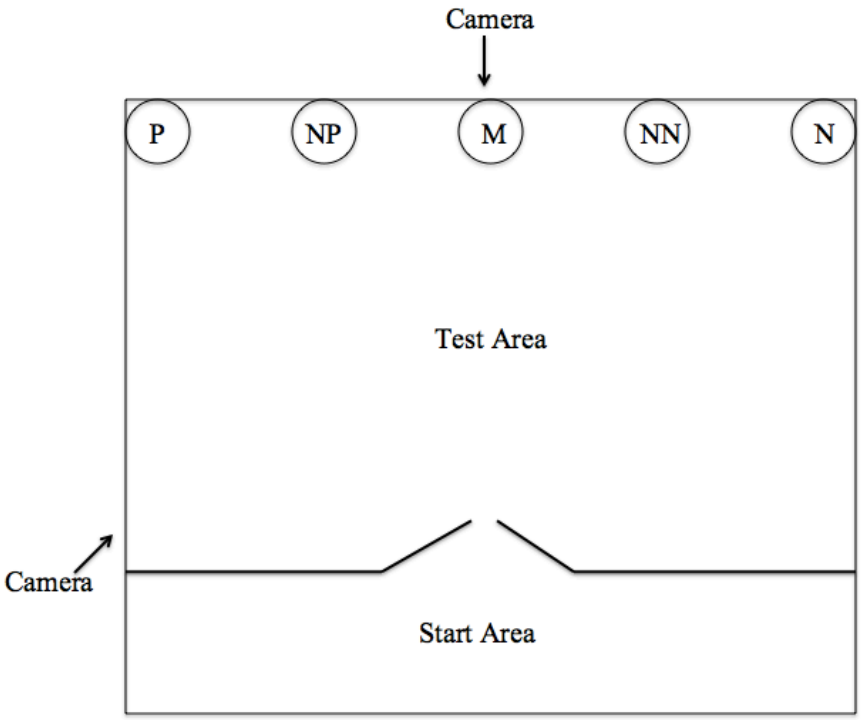


Figure 2: Experiment set up for the training, cognitive bias test and reminder trials. Probe locations show where each bowl would be placed for an individual that had been trained to run to the left corner and avoid the right.

2.2.4 Memory

As mentioned in section 2.2.3, each pig received a five trial 'reminder' session of alternating reference probes (P, N, P, N, P) the day before cognitive bias testing sessions. Whilst this acted as a reminder of the probe locations for the pigs between sessions, it was also used to test memory. Each pig's responses to the P and N locations was recorded as correct or incorrect, with correct responses being to approach the P bowl during the P trials within 30 seconds, and not touching the N bowl during the N trials. As this was done prior

to each test session, it allows for any changes in responses to be tracked throughout the gestational period. A binary record was also kept for the number of time a pig approached the positive corner before the negative during a negative trial.

2.2.5 Puzzle testing

Carried out similarly to the novel object tests, each individual was given a puzzle containing chocolate at the before, early and late stage of gestation. These puzzles consisted of three differently shaped wooden boxes with three different opening mechanisms, one with a flip lid, one with a sliding lid and one with a swivel lid. These were designed to look and work differently, requiring different manipulations for opening, while maintaining the same level of difficulty. A maximum of 120s was allowed for the individual to approach the puzzle. Once the pig approached the puzzle, it had a maximum of 300s was allowed to solve it. Puzzle order was counterbalanced between pig and test session.

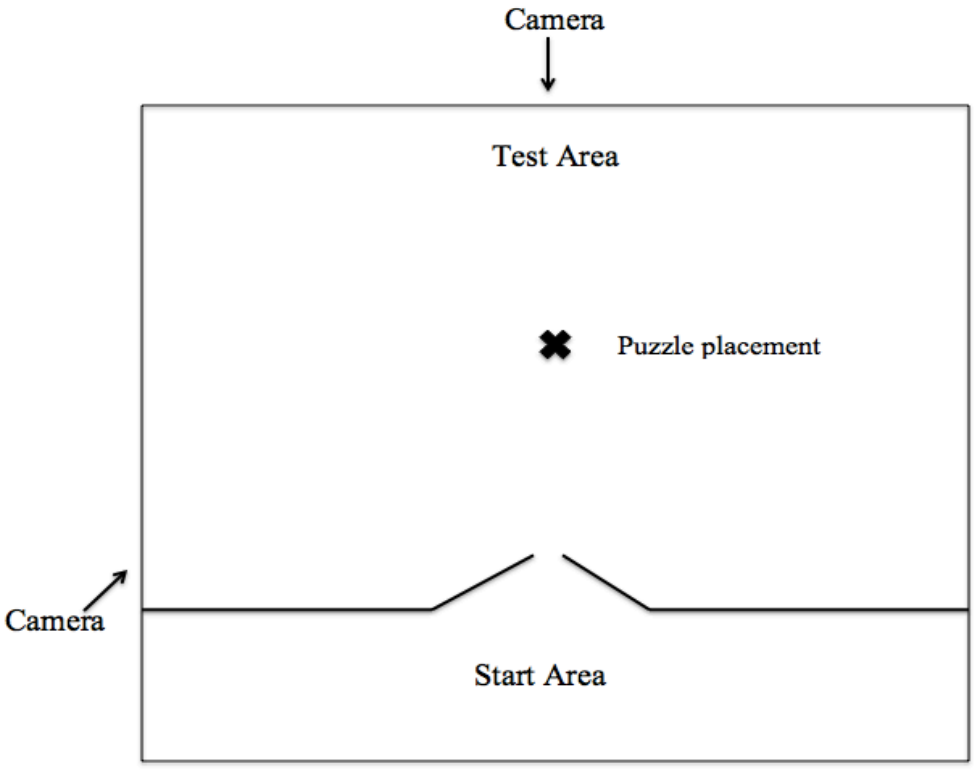


Figure 3: Experimental set up for the problem solving test, where the puzzle would be placed in the middle of the testing area.

2.2.6 Video analysis

The video footage of the test trials was used to record the movements of each gilt during each different test. The latency for each gilt to reach the bowl presented in the cognitive bias test and preceding reminder trials was measured. If the pig did not approach the bowl during the trial the latency was recorded as 30 seconds. The social isolation, novel object and puzzle tests were analysed to account for the time spent walking and running, standing, exploring the floors and walls as well as the time taken to approach the novel object or puzzle, the time spent investigating the novel object or puzzle and the time take to solve the puzzle. All latencies were measured in seconds (s).

2.2.7 Statistical analysis

Data was analysed using R Studio v. 1.0.136 (RStudio Team, 2016). To calculate the personality of each individual the method by Friel, *et al*. (2016) should be used however due to the small sample size of this study that was not possible. As an alternative, z-scores were created for the mean time to approach the novel object before pregnancy and the mean time to approach the novel object and puzzle during the early and late pregnancy test times. Each pig was assigned to either 'proactive' or 'reactive' depending on their overall z-score, with negative z-scores considered as more proactive personalities and positive z-scores considered more reactive. ANOVA tests were used to compare any differences in personality scores across test session and pig, as well as to compare the three puzzles used to ensure they were of the same difficulty.

General linear mixed models using lmer from the lmeTest package in R (Kuznetsova, *et al*., 2016) were used for all other analyses, with the final minimum adequate model created using stepwise deletion. For the cognitive bias analysis, time to approach the probe was the response, personality (Proactive or reactive), probe location and test time (early or late gestation) were all included as explanatory terms and pig ID was included as a random

effect. Probe location was included as a continuous variable, with N assigned to 1 and P to 5, as this gave us more power to uncover significant differences in the pigs' responses given the small sample size. To investigate memory, the 5 trial reminder sessions were used. The positive and negative probe locations were analysed separately with time to approach the location as the outcome variable, pig included as a random effect and personality and test time were included as covariates. In the model analysing the negative trials, whether the individual approached the positive location first was also included as a factor. Finally, to analyse the puzzle tests, time to solve the puzzle was the outcome variable, pig included as a random effect and personality, test time and time to approach the puzzle were included as covariates.

For the cognitive bias analysis, initial data exploration suggested that the responses of pigs in the before and early gestation groups, and those in the middle and late groups were very similar. Therefore, the before and early, and middle and late test times were combined into "early" and "late". For all analyses, the residuals of the models were examined to ensure that they conformed to assumptions of normality.

2.2.8 Ethics statement

This study was ethically approved by the University of Lincoln's ethical committee in December 2016 and given the approval ID CoSREC262.

2.3 Results

2.3.1 Personality

In total there were five reactive and five proactive pigs and each individual's personality did not change throughout pregnancy ($F_{1,3}$= 0.075, p= 0.786).

2.3.2 Cognitive bias

Pigs before and during early gestation had significantly more optimistic responses to the ambiguous NP, M and NN probes than during middle and late pregnancy ($F_{1,366}$= 5.1389, p= 0.0239801) (figure 4). The location of the ambiguous probe determined the pig's response at each test time, with the middle location resulting in the most varied latencies across each test time ($F_{1,366}$=11.7792, p= 0.00067). The pig's personalities influenced their responses in the cognitive bias test between test times ($F_{1,8}$= 2.9135, p=0.034356).

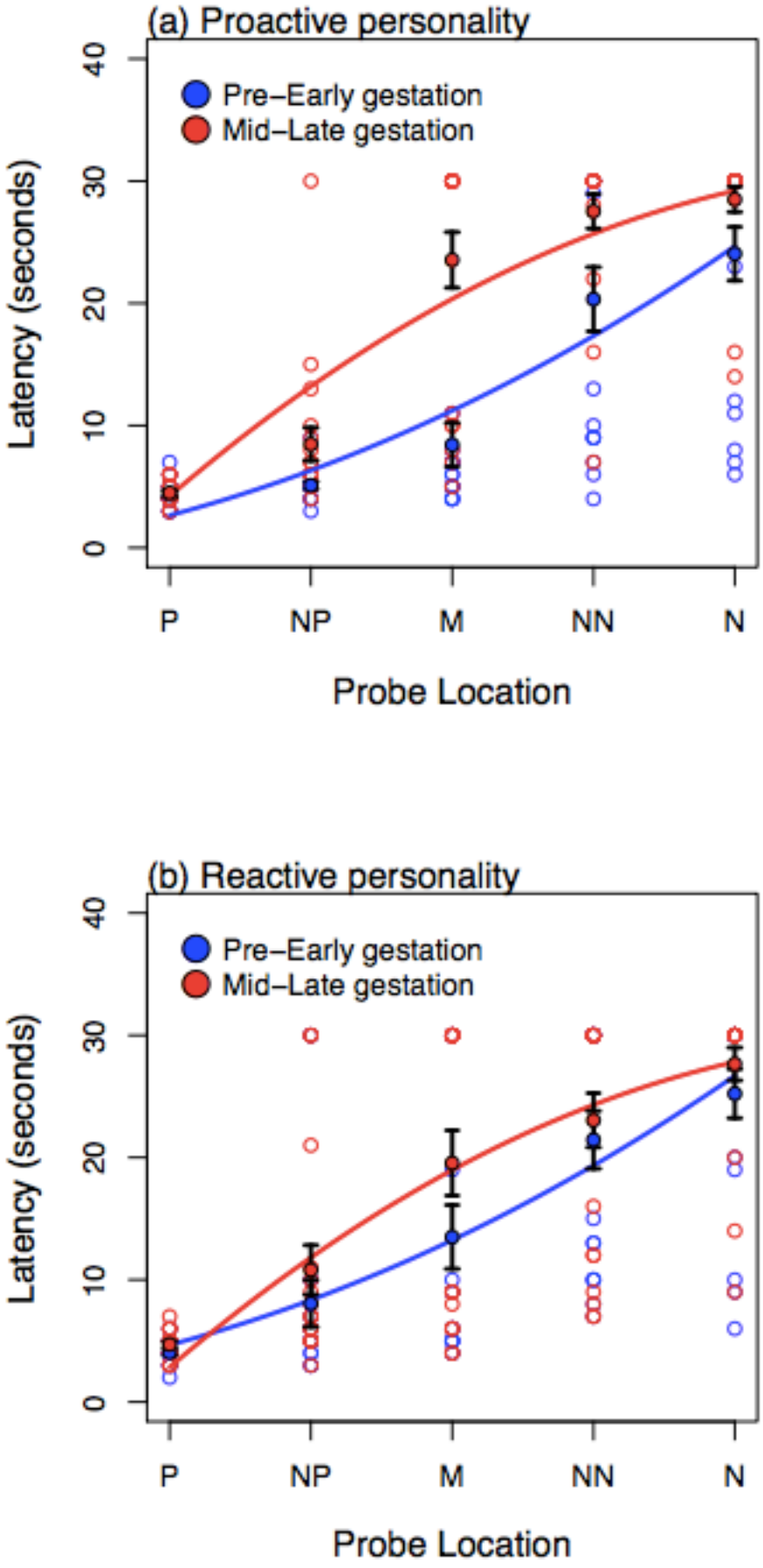


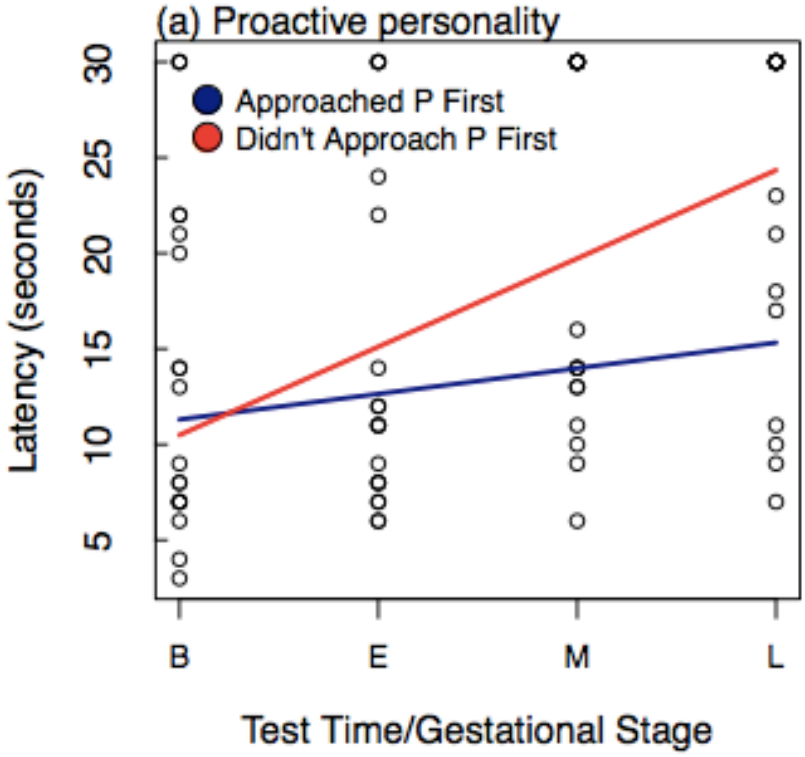
Figure 4: Latency to approach each location for two different test times; pre-early gestation (before and 5 week responses combined) and mid-late gestation (8 and 11 week responses combined). (a) Shows the responses for all 5 proactive pigs and (b) shows the responses for the 5 reactive gilts.

2.3.3 Problem-solving

Different puzzle designs did not differ in time taken to solve them ($F_{1,2}$= 0.360, p= 0.783). The pigs became faster to solve the puzzles throughout pregnancy with the fastest times occurring during the late stage ($F_{1,16}$= 4.8582, p=0.0429). The gilts became slightly faster to approach the puzzle towards the later stages of gestation ($F_{1,23}$=3.9932, p=0.0576) however personality did not affect the time taken to solve the puzzles at any stage of gestation.

2.3.4 Memory

During the reminder trials, as expected, the time to reach the rewarded P location did not change significantly throughout pregnancy ($F_{1,168}$=0.18064, P= 0.671). The pigs got slower to approach the N location throughout pregnancy with the slowest latencies occurring during the late stage of pregnancy ($F_{1,66}$=11.4254, p=6.66e-05). A significant interaction between the pig's personalities and whether the individual ran to the empty P corner first during the N trials was also found ($F_{1,67}$=5.6979, p=0.0198) (Figure 6).



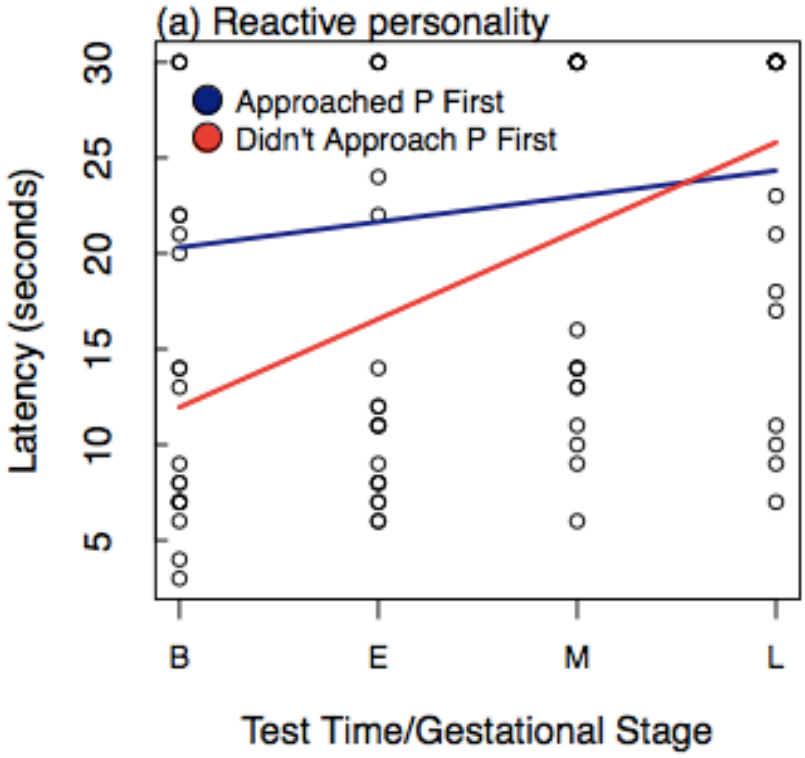


Figure 6: Latency for each individual to approach the N reminder trials, and whether the P location was approached first, at four different stages of the pig's 16-week gestational period; before, early (5 weeks), middle (8 weeks) and late (11 weeks). Figure a) shows responses of the five pigs with a more proactive personality type and figure b) shows the responses of the five pigs with a more reactive personality type.

2.4 Discussion

The cognitive bias of 10 gilts was tested at four different stages of gestation and it was found that there was an increase in latency to reach the ambiguous NP, M and NN test probes between before/early and middle/late pregnancy (figure 4). This suggests a shift in the pig's mood state, and that they may become increasingly pessimistic towards the latter stages of pregnancy. Time to approach each location increased as they reach the later stages of pregnancy. This result supports the hypothesis that pregnancy would influence the pig's cognitive bias and that cognitive bias would depend on the stage of pregnancy. The average latency to reach the positive and negative reference probes remained constant across test times (figure 4), again suggesting that the ambiguous stimuli were judged more pessimistically during the later stages of pregnancy, most likely due to a change in the gilt's mental state. This is the first study, to my knowledge, to show the effect pregnancy may have on the mood/mental state of a non-human animal and reflects findings in human females that mood and cognitive changes are most prevalent during the second or third trimester (Bennett, *et al*., 2004).

It may be suggested that other factors may be the cause of this finding, for example it may be that the increased latency is the result of learning that the ambiguous locations do no contain a reward. This was highlighted by Doyle, *et al*., (2010) who showed that sheep over a period of three weeks took longer to approach each of the ambiguous locations, despite there being no change to the animals environment showing that cognitive bias testing may not be very repeatable. In this case of this study, if learning were solely responsible for these results it would be expected to see the latency increase each successive time the animals were exposed to the unrewarded test probes, which was not the case. Another possible query could be whether the gilts got slower as they became more heavily pregnant. This was assessed by monitoring the time to approach the reinforced interim P and N trials during testing, and found there to be no difference across

the time period within individuals, suggesting that the pigs did not get slower due to any physical limitations during pregnancy. However, it is possible that the pigs overcompensated to run faster to reach the positive location they knew contains a reward and so the slowing effect of pregnancy was only visible at the ambiguous locations.

One limitation of this cognitive bias study set up is that the locations are not all equidistant from the starting position. This could impact upon results as it may mean that the pigs perceive different locations with different 'costs' depending on how far away they are. It also meant that even if the individual moved at the same pace to each location, it would take them slightly longer to reach the near negative and near positive than it would the middle location, potentially affecting the results of this study. In future studies, it would be preferable if the experimental set up meant that the locations were equidistant from the starting location.

Personality scores did not change significantly during pregnancy, supporting hypothesis (iv). This was expected as personality is defined as a set of consistent individual differences in behaviour across time and contexts (Sih, *et al*., 2004). This is also the first study to my knowledge to investigate the moderating effects of personality on the cognitive impacts of pregnancy on an individual. Personality had a significant influence on the cognitive bias outcome. In a previous study, Asher, *et al*. (2016) also found that personality and context interact to determine the cognitive bias outcome, however this was using weaner/grower pigs. Our results showed that proactive individuals had a larger change in latency between the before/early and middle/late test times in comparison to the reactive gilts. This may suggest that proactive individuals had a larger change in mood between the stages of gestation. It could also be due to the difference in coping styles and it is possible that reactive individuals coped better with the changes in mood and therefore did not show such a difference between how the ambiguous locations were interpreted at different test times. It should also be noted that the social isolation and novel object tests

used to create the personality scores were always done in the same order. This may have impacted upon the results as the pigs would know to expect the NO test after the SI test and could have adapted their behaviour.

It was hypothesised that memory would be altered during pregnancy relative to memory before pregnancy and I investigated this by analysing the 5-trial reminder sequence that each pig carried out the day before the cognitive bias test. When analysing at the positive trials there was no significant difference in the latency across test times. This was to be expected as this location was reinforced with a reward. On the other hand, the negative trials had a significant difference in latency between test times, showing that the pigs took increasingly longer to approach this location as they reached the later stages of pregnancy. This indicates that the pigs learnt and remembered that the negative bowl contained undesirable coffee beans and although it cannot be concluded that memory improved, this result does suggest that memory did not decline as a result of pregnancy. This finding contradicts much of the human and rat research in this area, which generally suggests that memory declines during pregnancy, if there is any change at all (Galea *et al.,* 2000; Keenan, *et al*., 1998). This area would be an interesting focus for future studies.

A binary record of whether the pig ran to the positive corner during a negative trial was kept for the reminder trials. This was analysed during the N reminder trial analysis and there was a significant interaction between whether the individual ran to the positive location first (despite there being no bowl there) and the individual's personality. This interaction shows a difference between personality types if the individual ran to the positive corner first, with more reactive type pigs taking longer to reach the negative location if they chose to run to the positive location first, whereas pigs with a more proactive personality type were faster to reach the negative corner than those who did not run to the positive location first (Figure 5). This difference in response between personality types is reflective of their definition with more proactive individuals being

more active and less flexible with their behaviour (Koolhaas, *et al*., 1999). These proactive characteristics may explain why these pigs did not change their behaviour during the negative trials even though no positive bowl was present.

The third hypothesis predicted that the pig's problem solving ability would change throughout pregnancy. A significant difference between test times was found as the time it took for each pig to solve the given puzzle decreased with each gestational stage. This suggests that the gilts problem-solving abilities improved throughout pregnancy and supports hypothesis (iv). Alternatively it is possible that the puzzles used to carry out this test were too simple and did not challenge the pigs appropriately and it is also possible that this was the result of learning due to repeat exposure to the puzzles. These factors should be taken into consideration when interpreting this result. Results also revealed that the individual's personality was not an influencing factor and that there was no significant difference in the difficulty of the puzzles.

2.5 Conclusions

It was found that pregnancy has a significant effect on cognitive bias in pigs with individuals taking increasingly longer to approach the ambiguous locations. This suggests that the gilts became increasingly pessimistic towards the end of pregnancy, however learning the bowls were unrewarded and the possible effect of pregnancy on the speed of the pigs should be considered as possible contributing factors. The pig's personality also influenced the cognitive bias outcome, with proactive individuals showing a more pronounced difference in their cognitive bias responses at different gestational stages. The pigs memory did not decline as a result of pregnancy, although it cannot be concluded that memory got better either. The gilts were faster to solve each puzzle at each subsequent test time, however caution should be taken when interpreting this as an improvement in problem solving as it is just as possible that this could be the result of learning. These

findings provide a basis for future work in this area, as well as highlighting the importance of considering the impact of large physiological changes, such as pregnancy, in animal cognition and welfare. If the changes in cognitive bias here are truly a result of a change in mood or affective state it is possible that this could not only effect the mother but also offspring as in-utero condition, such as prenatal stress, has been shown to change offspring's behaviour (Jarvis, *et al*., 2006), response to stress (Davis, *et al*., 2011) and even health (Merlot, *et al*., 2015; Merlot, *et al.*, 2008). It is possible that the potential change in cognitive bias during pregnancy could be eased by practical actions on farm, such as by providing suitable enrichment and space for gestating sows and gilts (Douglas, *et al*., 2012).

Chapter 3 - Effects of early life factors on cognition and health

Abstract

In utero and early life factors, such as litter size and sex ratio, have the potential to influence long-term health, welfare and behaviour. Previous research has shown how factors such as inter-uterine crowding, litter size, sex ratio and the perinatal environment can influence health and cognitive processes into adulthood, in both humans and non-human animals. Sex ratio and litter size as possible early life influences on cognitive bias and physical injury scores was investigated. It was found that cognitive bias was related to litter sex ratio; individuals originating from a litter with a higher ratio of males:females took more time to reach all probe locations than pigs from a more female biased birth litter, regardless of the sex of the responder. Body injury scores were associated with an interaction between the pig's relative weight and the litter sex ratio. Tail injury scores were associated with the pig's relative weight and litter size and, overall, female individuals had higher body and tail injury scores than males. Results showed that smaller litters were more likely to be male biased, a result that correlates with previous findings from other studies. These results show that the size and sex ratio of a litter have the potential to impact on the performance and welfare of that individual into adulthood, with real life implications for farmers in terms of health and welfare.

3.1 Introduction

From the moment of conception, the environment has the potential to effect long-term health, physiological and behavioural traits (Gluckman, *et al*., 2008; Hanson & Gluckman, 2008; Tang & Verstynen, 2002; Nyman, 1967). In humans and other mammals, plastic foetal programing can cause changes that last a lifetime, including how an individual responds to stress (Davis, *et al*., 2011; Glover, *et al*., 2010; Darnaudéry & Maccari, 2008, Gicquel, *et al*., 2008), its behaviour (Bale, *et al*., 2010; Kofman, 2002; Lemaire, *et al*., 2000), as well as its health and disease risk in adulthood (Harris & Seckl, 2011; Warner & Ozanne, 2010; Seckl, *et al.,* 2007). For mammals that give birth to litters of offspring, the effects of multiple siblings developing in the same womb space is a further potential environment influencer.

Inter-uterine position and the sex of adjacent littermates *in utero* has been shown to affect the individual's performance as an adult including their reproductive performance, behaviour and cognition with the potential to impact further future generations (mixed mammals: Ryan & Vandenbergh, 2002; pigs: Drickamer, *et al.,* 1997; mice: Vandenbergh & Huggett, 1994; humans: McFadden, 1993). For example, in gerbils hippocampal size can vary depending on sex and sex of neighbouring siblings in utero (Sherry, *et al*., 1996). Testosterone levels are higher in individual foetuses developing between two males, than in individuals developing between two females, regardless of the sex of the individual itself (Clark, *et al*., 1991). Males adjacent to other males *in utero* have larger scent glands and engage in more scent marking activity, whereas dams situated between two males *in utero* were more likely to produce litters with a greater proportion of males than females (Clark & Galef, 1995; Clark, *et al*., 1992; Clark, *et al*., 1990).

The size of the litter *in utero* can also have a long-term effect on health. In pigs, due to a sow's limited uterine space, individuals carrying large litters of piglets are at risk of intra-

uterine crowding, which can affect the performance and viability of offspring (Bérard *et al*., 2010). Increased litter size has been shown to negatively correlate with birth weight and size, and pre-weaning weight gain, and is also linked to increased variability in weights of piglets (Quiniou, *et al*., 2002; Wilsson & Sundgren, 1998). Piglets from a larger litter experience more competition for access to teats, are generally smaller and weaker at birth, and have poorer thermoregulatory abilities, making them less viable and at an increased risk of crushing, starvation and other causes of mortality (Herpin, *et al*., 2002; Andersen, *et al*., 2000; Auldist, *et al.*, 1998). Overall there is a large amount of evidence suggesting that larger litters have negative health and performance outcomes, which may also have negative consequences for the piglet's overall welfare (Rutherford, *et al*., 2013).

Litter size has been linked to sex ratio, with smaller litters tending to be male-biased, and larger litters female-biased (Servanty, *et al*., 2007; Peaker & Taylor, 1996). In many livestock production systems this has a potential economic impact, as females are the output-generating sex (e.g. producing eggs, milk, or as breeders). More broadly, sex ratio and the drivers for sex ratio bias (Fisher, 1999) have received considerable attention in the field of ecology and evolutionary biology (Trivers, & Willard, 1973; Tesfu, *et al*., 2014; Cameron, *et al*., 2008; Berry & Cromie, 2006; Grant *et al*., 2007).

Sex ratio bias has been shown to have an effect on processes and behaviours at an individual level (Darnaudéry & Maccari, 2008; Wilsson & Sundgren, 1998). One example of this is aggressive behaviour in pigs, which D'Eath & Lawrence (2004) found was more prominent in individuals originating from large litters, a result previously reported in rats (Seitz, et al., 1954) and thought to be due to the increased competition for teats during time spent in the litter (D'Eath & Lawrence, 2004). Aggression in pigs often results in some level of body injury, including scrapes and scratches, which can be measured using body injury scores. Injury can occur due to aggressive behaviour as the result of mixing unfamiliar individuals into a group or establishing a dominance or hierarchy within the

group (Stukenborg, *et al*., 2011; D'eath, 2005). Injury due to aggression has not only the potential to impact upon welfare due to the increased stress, disease and physical illness but also the overall production performance due to aggressive encounters increasing the likelihood of mortality and therefore economic loss (Marchant-Forde & Marchant-Forde, 2005; Held, *et al*., 2002).

A limited amount of evidence suggests that litter size and sex ratio of the litter can impact on long-term cognitive performance, such as Galea *et al.* (1994) who found that litter sex ratio in rats may influence spatial performance into adulthood. Using a spatial hole-board test Gieling *et al*. (2012) found that low birth weight pigs from larger litters had mild memory impairment. This study defined low birth weight as one standard deviation below the average weight of the litter, however later Antonides, *et al*., (2015) repeated this study with stricter criteria. This involved using previous weight data to estimate low birth weight as being one standard deviation below the average weight of nearly 500 other piglets. Following this it was found that, in contrast to the original study, low birth weight piglets had enhanced memory (Antonides, *et al*., 2015). Similarly, Fijn, *et al*., (2016) found that litter size did not affect piglets' emotionality as determined by an open field test, however, in adult male rats it has been shown that litter size may impact emotionality responses (Dimitsantos, *et al*., 2007).

More frequently cognitive, or judgement bias, tests are being used to infer affect, mood state and emotion in non-human animals. This is based the concept that cognition can be influenced by mood and emotions, causing attention, judgment and memory to be altered in the short, or even long term (Boissy, *et al*., 2007) and that happy, content individuals are more likely to make positive assumptions about ambiguous stimuli (Bethall, *et al*., 2015). Already applied to a variety of different species (Asher *et al*., 2016; Brydges, *et al*., 2011; Mendl, et al., 2010; Bateson & Matheson, 2007) this type of is easily adapted and

has shown how different environment factors can influence cognitive processing and mood state in non-humans animals.

There is currently no information relating production performance (e.g. in terms of injury scores) and litter qualities such as size and sex ratio, or indeed what potential impacts these litter qualities may have on cognitive bias in littermates in later life. The aim of this study was to investigate possible influences of early life factors litter size and sex ratio on cognitive bias and injury scores in later life. Three key hypotheses were tested:

(i) Litter size will be associated with the sex ratio of the litter

(ii) The litter size and sex ratio in the litter will be associated with an individual's cognitive bias

(iii) Litter size and sex ratio in the litter will be associated with body and tail injury scores

3.2 Method

The methods outlined in this chapter were originally conducted as part of BBSRC grant BB/K00254/1 and BB/K00254/2 between 2013 and 2016. This author was not involved in the original data collection, but conducted statistical analysis of the dataset as outlined in section 3.2.4. A description of the methods is outlined below for clarity and comprehensiveness, but full descriptions can be found in Asher et al (2016), Friel et al (2016) and Stevens et al (2017).

3.2.1 Animals and housing

Commercial crossbreed PIC337 (Large White x Landrace) pigs were weaned at four weeks and assigned to one of four pens consisting of 18 pigs balanced for sex and weight. As standard each pen had a slatted area within solid floors and wooden blocks on chains as

basic enrichment, however pen one and three were classed as high-level enrichment with deep straw bedding and 2.18m × 5.16m in dimension, whereas pens two and four contained no straw bedding and were smaller (2.18m × 3.42m) and were therefore classed as low-level enrichment. Each pig remained in their assigned pen for approximately six weeks and had access to standard weaner diet and water ad libitum. All pigs were weighed at 4 and 10 weeks of age, this was used to calculate the relative weight within each pen and replication. This was used as previous research suggests that relative size in relation to an individuals pen mates may be more important than actual size (Stevens, *et al*., 2017; Nettle, *et al*., 2013). 71 individuals were selected to be personality tested and 36 of these pigs (24 males and 12 females) were also selected to be cognitive bias tested. Of the 36 individuals which were cognitive bias and personality tested, there was litter size and sex ratio data available for 27 of these pigs. These 27 pigs were used for this study's analysis.

3.2.2 Cognitive Bias

Cognitive bias training and testing occurred between seven and ten weeks of age. Each individual was habituated to the testing area before training began. Pigs were trained to associate a positive or negative outcome depending on bowl location. The positive (P) location was situated in one corner of the experimental room and contained a reward of three sugar-coated chocolate sweets whereas the negative (N) location in the opposite corner contained three bitter coffee beans. A false-bottomed bowl containing three coffee beans and three sugar-coated chocolates was used to minimise olfactory cues, and the locations were pseudo-randomised and counterbalanced over environmental treatments for each individual. Training involved one bowl present per trial in alternating P and N locations before progressing to 5P and 4N in a randomised order. In order to continue onto the testing phase each individual was required to reach an 80% 'success' criteria, defined by approaching the P location within 30 seconds and not approaching the N location in 30 seconds. All individuals reached the criterion, except nine individuals that failed to

habituate and were excluded, giving a testing population of 17 males and 10 females from both the more enriched (n=12) and less enriched (n=15) environments. Tests included three unrewarded ambiguous test probe locations (near positive, NP; middle, M; near negative, NN) presented between the P and N locations resulting in nine trials per test (e.g. P, N, M, N, P, NN, P, N, NP). Two tests were conducted per individual and pigs were given 30 seconds to approach each probe before being returned to the start box. If the pig did not approach the bowl during the trial the latency was recorded as 30 seconds

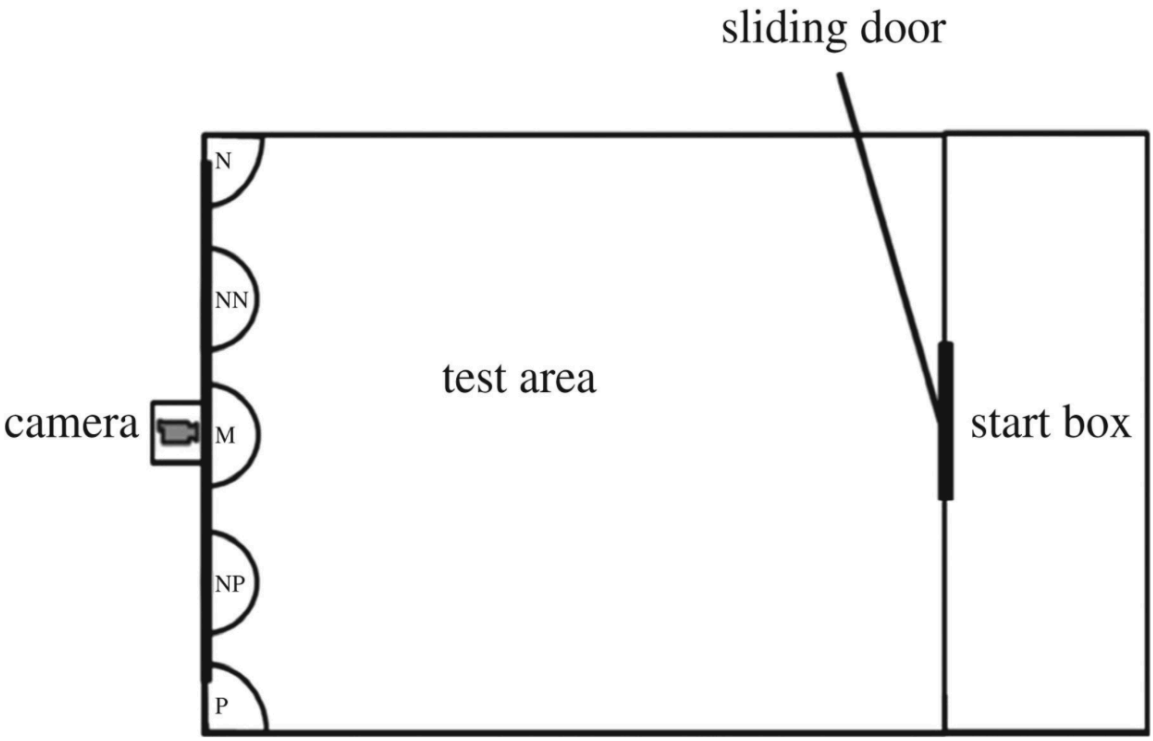


Figure 7: Room layout for the cognitive bias test and training. Positive (P) is a rewarded location and negative (N), near negative (NN), middle (M) and near positive (NP) are unrewarded. Sourced from Asher, *et al*. (2016)

3.2.3 Personality Testing

Each individual undertook a novel object (NO) and social isolation test (SI). An orange traffic cone and a white bucket were used as novel objects. These were counterbalanced for environment and pseudo-randomised across tests (only one was presented per test). Each pig was given 2 minutes to enter the arena (l × w × h: 3.6 × 2×1.2 m) and following

this the novel object was lowered into the arena on a rope until it was approximately 10cm from the ground. This signalled the start of the novel object test, which lasted 5 minutes. For the social isolation test each individual was placed in a pen (l × w × h: 2.2 × 1.7 × 1.2 m) away from his or her home pen for 3 minutes.

Video cameras were used to record each test from above, including the time spend standing, exploring, moving and line crossing in both tests. Latency to approach and maintain contact with the NO was also recorded. Both test areas were cleaned between tests and deep cleaned between testing pigs from different pens. Between pens pigs were tested in a randomly and within pens individuals were tested sequentially to minimise and stress and disruption experienced by the rest of the pen.

The final personality scores were calculated according to the method outlined in Friel, *et* al., (2016). From the behaviours measured in the novel object and social isolation tests, it was calculated which ones were repeatable using the intraclass correlation coefficient. The personality score was calculated as the mean of the z-scores of those repeatable behavioural measures (duration standing and exploring in the SI tests; and duration of standing, exploring and latency to approach the NO in the NO tests) with Cronbach's alpha as a measure of internal constancy. A more detailed method is also outlined in Asher, *et al*. (2016).

3.2.4 Body Scores

Pigs were individually scored depending on their injuries according to a six-point system based on Conte, et al., (2012), at three different time points; day 4 post-mixing, between day 8 – 17 post-mixing and between day 29 – 39 post-mixing. These time-points were selected as they represented the time just after mixing, approximately one-two weeks later (when the group should be settled) and then toward the end of the study period, when the pigs are close to the time to move into the grower unit (Stevens et al., 2017).

Each individual was assessed on injuries on the ears, snout, shoulders, legs, flanks, hindquarters and back. Tails were also scored, although these were docked as part of the farm's standard practice. For this study the tail injury score and mean body score were used. Complete litter and body score information was available for 71 individuals, which were subsequently used in this study's analysis.

3.2.5 Statistical analysis

For this study, the sex ratio of a litter was determined by the percentage of males within the litter. All data analysis for this study was done using R Studio v. 1.0.136 (RStudio Team, 2016). Cognitive bias and corresponding litter data was analysed using R code adapted from Asher, *et al.* (2016). A general linear mixed model using lmer from the lmeTest package in R (Kuznetsova, *et al*., 2016) was used with latency to approach the probe as the response variable and replicate and pen included as random effects. Personality score, treatment, litter size and percentage males were all included as explanatory variables and the final model was created using stepwise deletion. Examination of the residuals form the model suggested deviation from normality and so the latency variable was logged and the analyses re-run. The residuals using the logged data conformed to the expectations of normality.

The body score and corresponding litter data were analysed using a general linear mixed model with mean body score as the response variable and litter size, percentage males, environment type, relative weight and sex all included as explanatory variables. Replicate was included as a random effect, as well litter ID nested within pen. The tail injury scores were all coded as a 1, 2 or 3 and so ordinal logistic regression was used for this analysis, using polr from the MASS package (Venables, *et al*., 2002). Tail injury score was the response variable with litter size, percentage of males, treatment, relative weight and sex included as explanatory variables.

Pearsons correlations between litter size and sex ratio were calculated using the cor.test function in R for all data sets.

3.2.6 Ethics statement

Data used in this chapter were retrieved from a study approved by the University of Lincoln's Ethics Committee (COSREC62 on 08/09/2015).

3.3 Results

3.3.1 Litter size and sex ratio

The size of the litter was significantly correlated with the number of males in the litter with smaller litters having a higher male: female ratio than larger litters, which tend to be more female biased. This was apparent in all of the data sets; cognitive bias (cor = -0.7093681, $p < 2.2e-16$), body score (cor= -0.3742328, $p < 2.2e-16$) and tail injury score (cor= -0.3725448, $p < 2.2e-16$).

3.3.2 Cognitive bias

The P and NP locations had the fastest latencies for all pigs whereas pigs were much slower to reach the NN and N locations ($F_{1,244}$=2.267, p=0.0005). Proactive pigs were also significantly faster to approach all five locations in comparison to reactive individuals ($F_{1,60}$=5.343, p=0.024). Responses to probes in the cognitive bias test was also affected by the percentage of males in the pigs birth litter, with individuals originating from a male biased litter being significantly slower to reach each location in comparison to female biased litters ($F_{1,76}$=12.250, p=0.0007).

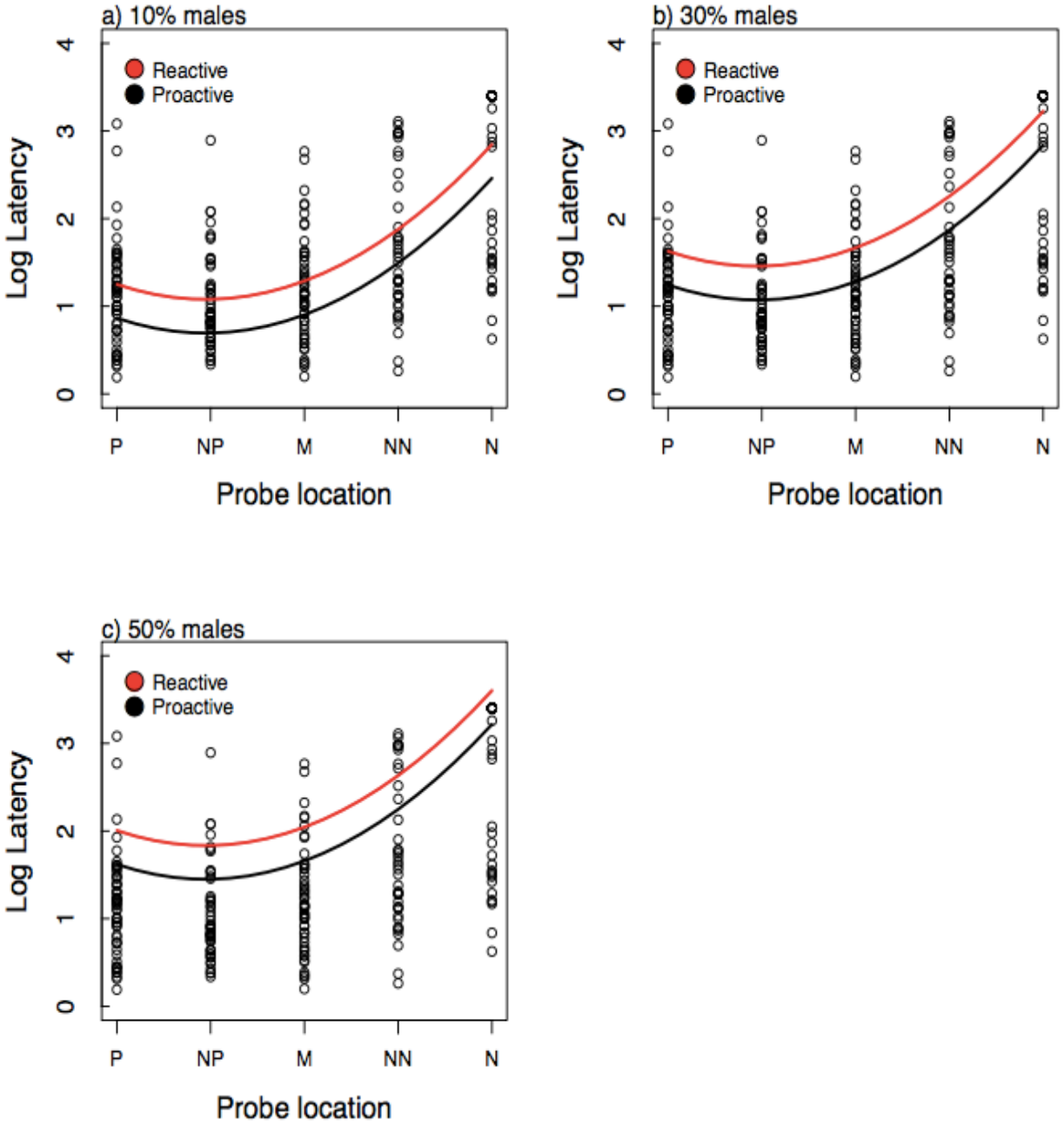


Figure 8: Latency to approach each probe location with more proactive pigs represented by the black line and more reactive individuals represented by the red line for litters with 10% (a), 30% (b) and 50% males (c).

3.3.3 Body injury scores

Female pigs sustained a higher mean body injury score in comparison to male individuals (Female mean = 1.623 (±0.439), Males, mean = 1.548 (±0.473); $F_{1,598}$= 4.3496, p=0.037). Pigs housed in an enriched environment were also more likely to have a higher body score

if they originated from a litter with a high male: female ratio. This was the opposite for pigs housed in a barren environment ($F_{1,599}$= 5.6721, p=0.017) (Figure 2, a & b). It was also found that there was a significant interaction between the treatment and pig's relative weight within the pen ($F_{1,598}$= 3.8765, p=0.049), showing that relatively heavy individuals in the enriched environment gained a higher body score than the lighter pigs, whereas the lighter pigs gained more injuries in the barren environment (Figure 2, c & d).

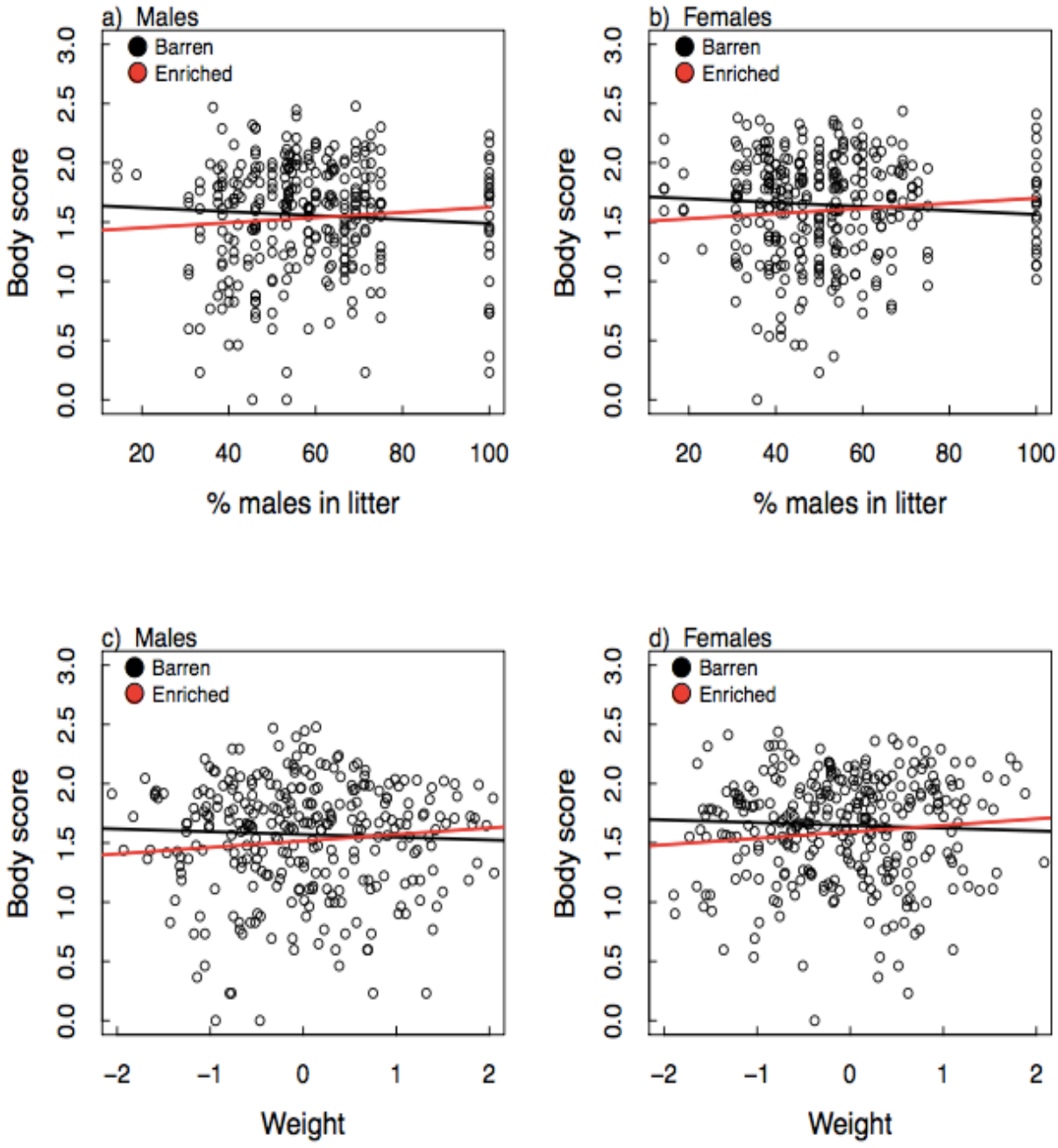


Figure 9: Mean body scores, with 0 representing no lesions and 3 being the maximum score, separated by sex, the percentage of males in the individual's birth litter (a and b) and relative weight (c and d).

3.3.4 Tail injury scores

The results from the ordinal logistic regression model looking at tail injury scores showed a significant effect of the pig's relative weight with the heaviest and lightest individuals within the groups sustaining the most tail injury (t= 2.224, p= 0.026). There was also an interaction between litter size and weight (t=-2.171, p=0.029), showing that within the lightest and heaviest pigs the individuals originating from a very large or very small litter had higher tail injury scores. There was also an interaction between treatment and relative weight (t=-2.818, p=0.004), as well as a more complicated three-way interaction between litter size, relative weight and pen treatment (t=3.185, p=0.001) showing that the lightest pigs had a higher tail injury score if they were in an enriched environment and originated from a small litter or were in a barren environment and originated from a larger litter (Figure 3a). Average weight pigs sustained the same amount of tail injury despite which environment they were in or what size litter they originated from (Figure3c), however heavier individuals from a large litter size gained more tail injury in the enriched environment. Relatively heavier individuals also gained more tail injury in the barren environment if they originated from a smaller litter (Figure 3b).

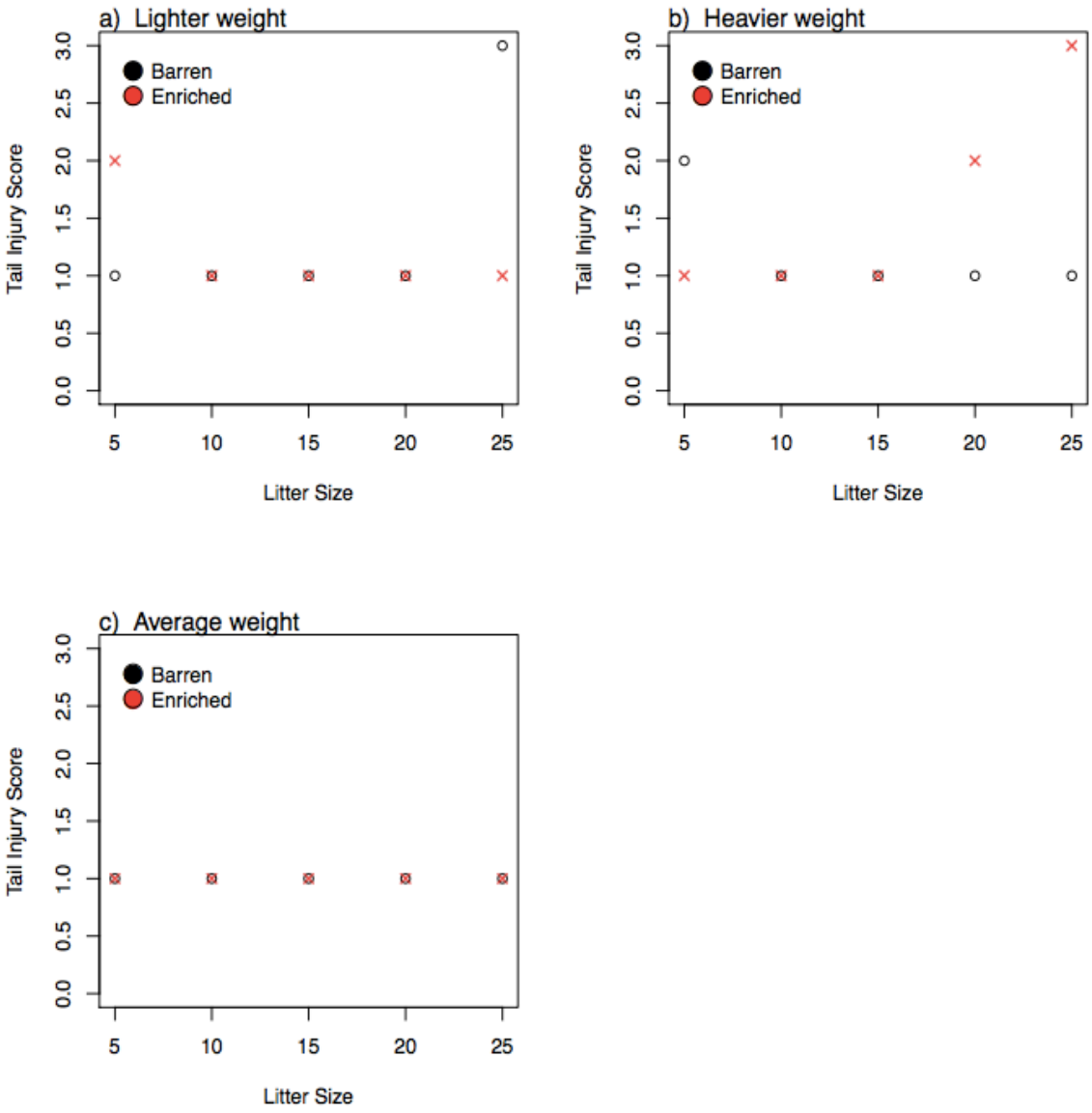


Figure 10: Tail injury score, with 0 representing no lesions and 3 being the maximum score, depending on litter size and pen treatment (red crosses - enriched and black circles - barren) for low (a), high (b) and average (c) relative weights.

3.4 Discussion

It was hypothesised that the litter size and sex ratio in the litter will be associated with an individual's cognitive bias. A link between litter size and cognitive bias was not found however the percentage of males in an individual's home litter was linked to their cognitive bias; individuals originating from more female-biased litters were quicker to

reach all probe locations overall in comparison to pigs from less biased litters (figure 1). This finding partially supports hypothesis (ii) and suggests that pigs from female-biased litters more likely to interpret ambiguous stimuli optimistically. This result highlights for the first time the influence sex ratio of an individual's home litter can have on cognitive bias in adulthood. One possible explanation for this finding may be that the sex ratio bias in utero impacts on circulating hormone levels during the gestation period, impacting on how the neural network structure develops (Bayless & Shah, 2016) and thus how individuals subsequently respond to different types of stimuli.

As expected, pigs with a more proactive personality were consistently quicker overall to reach all locations in comparison to pigs with a more reactive personality type. These differences are typical of the two personality types, with more proactive personalities tending towards more active and less flexible behaviour (Sih, *et al*., 2004; Koolhaas, *et al*., 1999). Although this result could also be interpreted as the more proactive individuals being more optimistic, it should be considered that they might just be faster overall in comparison to the more reactive individuals. This trend in personalities was consistent throughout changes in litter size and sex ratio.

As predicted in hypothesis (i), there was a strong correlation between the size and sex ratio of a litter, with smaller litters more likely to be male-biased than larger litters, which are likely to be female- biased. This result reflects other research, such as Servanty, *et al*. (2007) and Gorecki (2003), which also found in wild boar and domestic pigs that smaller litter sizes tended to be male- biased.

Analysis of the mean body injury scores showed that females are more likely to have a high body injury score. A significant interaction between the pen treatment and sex ratio was discovered, with pigs originating from a male-biased litter likely to have more body injuries in the enriched environment, whereas pigs born into litters with a lower percentage

of males have more injuries in the barren environment. This likely is related to the next finding, which is a significant interaction between the treatment and the pig's relative weight. The heavier the pig in the enriched environment the more likely it is to have more injuries whereas in the barren environment, a pig with a below average weight is more likely to have more injuries. These two findings may suggest a link between sex ratio and body weight, as male biased litters tend to be smaller in size, potentially allowing for each piglet to be heavier due to less crowding in-utero (Bérard *et al*., 2010). Heavier pigs also have an advantage over their lighter pen-mates and tend to be the most dominant in the group (Andersen, *et al*., 2000). There was a complex relationship between relative weight, level of tail injury and litter size, showing that only the relatively heaviest and lightest individuals in the pen that originated from the largest and smallest litters had an above average tail injury score. This is demonstrated in a significant 3-way interaction between litter size, relative weight and pen treatment (figure 3). Overall these results support hypothesis (iii) that litter size and percentage of males in the litter will be associated with body and tail injury scores.

This interaction shows that the effects of relative weight and litter size are apparent in the enriched environment pens, with males and females in enriched pens showing positive associations between percentage of males in the litter and body or tail score, and also relative weight and body or tail score. It is not possible to deduce the cause of these associations from the current dataset, however, these results suggest a number of testable hypotheses. The body or tail scores of pigs from enriched environments may have greater sensitivity to litter sex ratio and relative weight because (i) pigs from male biased litters have a different frequency or quality of aggressive behaviour compared with pigs from female biased litters. This is more apparent in enriched environments where there are additional resources to compete over; (ii) pigs with higher relative weight have higher body or tail scores as they have a different frequency or quality of aggressive behaviour

compared with pigs of lower relative weight. Again, this may be more apparent in enriched environments where there are more resources to compete over. Alternatively, (iii) pigs from enriched environments may have higher body scores as they have slightly more space and resources, and so are generally more active. Higher activity levels may result in higher body scores, even if that activity is not aggressive. As tail biting is not likely to simply be associated with higher activity levels per se (as it is considered to be driven by a different motivation to body-directed injuries), one may hypothesise that if (iii) was the case, then the relationship between tail scores and relative weight and percentage of males in the litter should be unaffected by treatment.

Overall, body scores are a quick, easy and non-invasive way to assess health of an individual, and can potentially act as a proxy for activity levels within a pen (Stephens *et al*. 2017). Body score analysis is often used as an indicator for aggression, with fights typically occurring within the first few weeks of mixing as a hierarchy is being established (Stukenborg, *et al*., 2011; Turner, *et al*., 2006). However, it should be remembered that it is possible to gain body lesions, and therefore a high body score, from everyday benign scrapes and scratches while being moved or due to a pen of highly active individuals, therefore this scoring may be best used as a marker for levels of activity rather than any particular behaviour, such as aggression. Tail injury scores are interesting to look at separately from the mean body score, as tail biting is a widespread unresolved issue in pig farming globally, and can be costly economically, as well as in terms of animal welfare (Schrøder-Petersen & Simonsen, 2001). The pigs in this study had their tails docked by up to 50%, this is a common method to try and reduce tail biting by sensitising the tail (Sutherland & Tucker, 2011). However, how tail docking may influence our results is unknown and in future studies it may be beneficial to assess pigs with intact tails (Stevens, *et al.,* 2017).

3.5 Conclusions

In conclusion, *in utero* litter size and sex ratio were shown to influence cognitive bias and body scores later in life. Results from this study showed that the sex ratio of an individual's home litter can influence cognitive bias responses with pigs originating from a female biased litter responding to the test probes significantly more optimistically. Litter size can influence tail injury scores in pigs below or above the average relative weight of the pen. Similarly, the sex ratio of an individual's home litter can influence body scores depending on the pen environment, for example pigs from a high male: female ratio litter gained a higher body score in the enriched environment than if they were housed in a barren environment. These results also showed that litter size and sex ratio are directly linked, with smaller litters tending to be male biased. These results show the influence that *in utero* and early life factors can have on outcomes into adulthood and have the potential to have real life implications for farmers in terms of terms of health and welfare. As farmers strive to continually increase litter size these results show how this may affect livestock after the pig has left the litter and placed into new and different environments.

Chapter 4 - General discussion

In this thesis, the main aim was to investigate the impact of pregnancy on cognitive processes and mood in primiparous gilts, and to investigate effects of prenatal and early life factors, including litter size and sex ratio, on cognition and health in pigs. It was found that pregnancy significantly alters cognitive bias responses, with individuals becoming increasingly pessimistic towards the later stages of pregnancy, suggesting a shift in the pig's mood state. As this bias appears to change depending on the stage of pregnancy that the animal is experiencing at that time, it may be suggested that fluctuating hormone levels are associated with these changes (Soldin, *et al.,* 2005), although this could not be concluded without further research. Hormone levels have previously been found to correlate closely with changes in mood, for example Buckwalter, *et al*. (1998) found that in pregnant humans, increased levels of progesterone correlated with greater mood disturbances, and progesterone is known to remain at high concentrations in pigs throughout pregnancy until the beginning of birth (Oliviero, *et al*., 2008; Ash, *et al.,* 1975). It is worth noting that whilst the housing environment for the gilts included in the experiment, described in Chapter 2, did not change during the course of the study, in production systems, pregnant sows would typically enter a farrowing crate for the final weeks before birth. Research on farrowing environments has identified confinement in the traditional crate as being a cause of poor welfare as it is not only physically restrictive but behaviourally, preventing behaviours such as nest building (Algers & Uvnäs-Moberg, 2007; Grandinson, 2005; Blackshaw, *et al*., 1994). In Chapter 3, pigs from female-biased litters were faster than pigs from male-biased litters to reach ambiguous stimuli in a cognitive bias test. Although this could be interpreted as being more optimistic, as they were faster overall to reach all locations, this may not be the correct interpretation. There are multiple potential hypotheses for why this could be the case; for example, female-biased litters tend to be larger, so it may be beneficial to be risk prone (or 'optimistic') if in early life resources were something to be competed for. An alternative might be that

the sex ratio bias in utero impacts on circulating hormone levels during the gestation period, impacting on how the neural network structure develops (Bayless & Shah, 2016) and thus how individuals subsequently respond to different types of stimuli. Although much of the research using cognitive bias tests focus on external factors both of these studies show that cognitive bias can be influenced by factors not related to external environment.

In both chapter two and three we also found that not only did cognitive bias change depending on the stage of pregnancy and whether the sex ratio of the litter pig originated from but that personality influenced the pig's responses in the cognitive bias tests. Previously personality has been shown to interact with mood to determine cognitive bias in weaner/grower pigs (Asher, *et al*., 2016) and our result shows that this is also true during gestation. In chapter two we found that reactive individuals had a less pronounced change in latency between the before/early and middle/late test times in comparison to more proactive individuals. This may be due to reactive pig's being better able to cope with physiological and mood changes. Subsequently in chapter three, personality was also a contributing factor in the cognitive bias test with proactive individuals being fast overall to reach the ambiguous locations. These results highlight how personality can be included in cognitive and behavioural testing to account for individual variation.

Further to cognitive bias, in chapter two it was investigated whether other aspects of cognition, specifically memory and problem solving, change over the gestation period. There was a significant difference in the pigs' individual responses to these tests during pregnancy compared with their performance pre-gestation. The five-trial cognitive bias probe reminder sequences was used to infer memory, this had the advantage of reducing the number of additional tests for the pigs, which also reduced unnecessary movement stress, whilst allowing us to assess memory at each stage of pregnancy. These results showed that memory did not decline as a result of pregnancy, although future research

may benefit from testing a non-pregnant control group. Including this control group would have allowed for comparison between the two groups, possibly allowing for the effect of pregnancy between test times to be distinguished from learning.

A test was designed to determine problem solving ability throughout pregnancy. The three puzzles used were designed to be simple, to ensure that the pigs would be able to solve them, as well as being similar enough to not vary in levels of difficulty without being the same, ensuring that each pig did a new puzzle at each testing stage. The results showed that all the pigs became quicker to solve the puzzles as their pregnancies progressed. Although piloted before the study started, it is possible that these puzzles were not adequate to test the aims in this study. To improve on this in future it may be beneficial for puzzles to have a transparent lid or panel so the individual doing the test can see what they are working towards. They could also be more challenging, with more difficult mechanisms to open the puzzle while being secure enough that they cannot be opened by accident.

In chapter three, as well as cognitive bias we investigated how early life factors; litter size and sex ratio, may impact upon body scores into adulthood. We found that these did early life factors influenced body and tail scores after the pig had left the litter. Body and tail injury scores are a quick, easy and non-invasive way to assess health of an individual, and can potentially act as a proxy for activity levels within a pen (Stephens *et al*. 2017).

Logical next steps to expand and improve both of the studies in this thesis would involve analysing other health conditions in relation to early life factors. Previous studies in rats and humans have shown how both the *in-utero* and early life environment can influence long-term health outcomes, such as obesity (Huang, *et al*., 2007; Breier, *et al*., 2001), heart and vascular health (Barker, 2002; Lamireau, *et al*., 2002), and general disease susceptibility (Cottrell & Seckl, 2009; Godfrey & Barker, 2000). Investigating how early

life factors or prenatal programming may influence common livestock diseases and illnesses would be an interesting next step. It would also be beneficial to investigate how other early-life or physiological factors may also affect cognitive bias or welfare in pig and other species. In light of the results in chapter 2 a logical next step would be to investigating further into the specific hormones and concentrations that may be playing key roles throughout the gilt's pregnancy. It would be beneficial to include a non-pregnant control group in chapter 2, as this would have allowed for comparisons in the cognitive bias test and during the memory and problem solving tests. This comparison would make it easier to assess how much other factors, such as learning, may be contributing to the results. Testing during the postpartum period would also make an interesting addition as some studies, such as De Groot, *et al*., (2006), have found that cognitive function continues to change post-partum, and likewise so does mood as, for example, Evans, *et al*., (2001) found depressive symptoms in humans are stronger after birth in comparison to during pregnancy. It should also be considered whether environmental conditions could improve the pigs' negative bias during late pregnancy, such as the addition of new enrichment during the later stages (Van de Weerd, *et al.,* 2003; Beattie, *et al*., 2000).

It may also be interesting to look at other aspects of cognition that may link the two studies done here. For example, recent studies in humans have shown that the sex of the fetus may influence the mother's cognition, such as a study by Vanston & Watson (2005) who found that women pregnant with male foetuses consistently out performed women carrying female foetuses in tests of spatial ability working memory. This would be an interesting study in a non-human animal, for example investigating how different sex ratios may influence maternal cognition. There has also been some research into foetal programing in pigs, typically focusing on maternal social stress (Jarvis, *et al.,* 2006; Otten, *et al.,* 2010), however it may be interesting to investigate links between the mother's cognitive bias through pregnancy as well as post-natally, with that of her offspring across its life course and, in particular, in response to typical stressors.

Overall conclusions

This thesis presents a series of novel results that advance the field of animal cognition and welfare, particularly in relation to the gestation period as experienced by both the mother and the offspring. The key results from these studies are that there is an association between stage of pregnancy and cognitive bias; and there is an interaction between sex ratio and litter size on offspring cognitive bias and injury scores. Overall, this thesis shows that early life factors and pregnancy can affect an individual's cognitive processing, which may have consequences in terms of the welfare as these results show that it is not just environmental factors that can influence mental state. This is an exciting finding, which has consequences for animal cognition research and livestock production. Understanding animal cognition and cognitive requirements may help us to continuously monitor welfare, with the potential for subsequently improving health and performance.

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References

Abbot, P. & Rokas, A. (2017) Mammalian pregnancy. *Current Biology*, 27, R127-R128.

Aiken, C.E. & Ozanne, S.E. (2013) Sex differences in developmental programming models. *Reproduction*, 145, R1-R13.

Aisa, B., Tordera, R., Lasheras, B., Del Río, J. & Ramírez, M.J., (2007) Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology*, 32, 256-266.

Algers, B. & Uvnäs-Moberg, K. (2007) Maternal Behaviour In Pigs. *Livestock Science*, 52, 78 – 85.

Allen, W.R., (2006) Ovulation, pregnancy, placentation and husbandry in the African elephant (Loxodonta africana). *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 361, 821-834.

Al-Qaraghouli, M. & Fang, Y.M.V. (2017) Effect of fetal sex on maternal and obstetric outcomes. *Frontiers in pediatrics*, *5*.

Andersen, I.L., Andenæs, H., Bøe, K.E., Jensen, P. & Bakken, M. (2000) The effects of weight asymmetry and resource distribution on aggression in groups of unacquainted pigs. *Applied Animal Behaviour Science*, 68, 107-120.

Anderson, M.V. & Rutherford, M.D., (2011) Recognition of novel faces after single exposure is enhanced during pregnancy. *Evolutionary Psychology*, 9, p.147470491100900107.

Anderson, M.V. & Rutherford, M.D. (2012) Cognitive Reorganization During Pregnancy And The Postpartum Period: An Evolutionary Perspective. *Evolutionary Psychology*, 10, 659 – 687.

Antonides, A., Schoonderwoerd, A.C., Nordquist, R.E. & van der Staay, F.J. (2015) Very low birth weight piglets show improved cognitive performance in the spatial cognitive holeboard task. *Frontiers in behavioral neuroscience*, *9*.

Arey, D.S. & Sancha, E.S. (1996) Behaviour and productivity of sows and piglets in a family system and in farrowing crates. *Applied Animal Behaviour Science*, 50, 135-145.

Arey, D.S., Petchey, A.M. & Fowler, V.R. (1991) The preparturient behaviour of sows in enriched pens and the effect of pre-formed nests. *Applied Animal Behaviour Science*, 31, 61-68.

Ash, R.W. & Heap, R.B., (1975) Oestrogen, progesterone and corticosteroid concentrations in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. *Journal of endocrinology*, *64*, 141-154.

Asher, L., Friel, M., Griffin, K. & Collins, L.M. (2016) Mood and personality interact to determine cognitive biases in pigs. *Biology letters*, 12, 20160402.

Auldist, D.E., Morrish, L., Eason, P. & King, R.H. (1998) The influence of litter size on milk production of sows. *Animal Science*, 67, 333-337.

Bale, T.L., Baram, T.Z., Brown, A.S., Goldstein, J.M., Insel, T.R., McCarthy, M.M., Nemeroff, C.B., Reyes, T.M., Simerly, R.B., Susser, E.S. & Nestler, E.J. (2010) Early life programming and neurodevelopmental disorders. *Biological psychiatry*, 68, 314-319.

Barker, D.J. (2002) Fetal programming of coronary heart disease. *TRENDS in Endocrinology & Metabolism*, 13, 364-368.

Bérard, J., Pardo, C.E., Béthaz, S., Kreuzer, M. & Bee, G. (2010) Intrauterine crowding decreases average birth weight and affects muscle fiber hyperplasia in piglets. *Journal of animal science*, 88, 3242-3250.

Bateson, M. and Matheson, S.M. (2007) Performance on a Categorisation Task Suggests That Removal Of Environmental Enrichment Induces 'Pessimism' In Captive European Starlings (Sturnus vulgaris). *Animal Welfare*, 16, 33-36.

Bateson, M., Desire, S., Gartside, S.E. and Wright, G.A. (2011) Agitated Honeybees Exhibit Pessimistic Cognitive Biases. *Current Biology*, 21, 1070-1073.

Baxter, E.M., Jarvis, S., Palarea-Albaladejo, J. & Edwards, S.A., (2012) The weaker sex? The propensity for male-biased piglet mortality. *PLoS One*, 7, e30318.

Bayless, D.W. & Shah, N.M. (2016) Genetic dissection of neural circuits underlying sexually dimorphic social behaviours. *Phil. Trans. R. Soc. B*, 371, p.20150109.

Behan, Á.T., Van den Hove, D.L., Mueller, L., Jetten, M.J., Steinbusch, H.W., Cotter, D.R. & Prickaerts, J. (2011) Evidence of female-specific glial deficits in the hippocampus in a mouse model of prenatal stress. *European Neuropsychopharmacology*, 21, 71-79.

Bennet, L. & Gunn, A.J. (2007) The fetal origins of adult mental illness. In *Early life origins of health and disease,* 573, 204-218

Bennett, H.A., Einarson, A., Taddio, A., Koren, G. and Einarson, T.R. (2004) Prevalence of depression during pregnancy: systematic review. *Obstetrics & Gynecology*, 103, 698-709.

Berry, D.P. and Cromie, A.R. (2006) Artificial insemination increases the probability of a male calf in dairy and beef cattle. *Theriogenology*, 67, 346-352.

Bethall, E.J. (2015) A "How-To" Guide For Designing Judgement Bias Studies To Assess Captive Animal Welfare. *Journal of Applied Animal Welfare Science*, 18, S18 – S42.

Blackshaw, J.K., Blackshaw, A.W., Thomas, F.J. & Newman, F.W. (1994) Comparison of behaviour patterns of sows and litters in a farrowing crate and a farrowing pen. *Applied Animal Behaviour Science*, 39*,* 281-295.

Bock, J., Murmu, M.S., Biala, Y., Weinstock, M. & Braun, K. (2011) Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats. *Neuroscience*, 193, 34-43.

Bock, J., Wainstock, T., Braun, K. & Segal, M., (2015) Stress in utero: prenatal programming of brain plasticity and cognition. *Biological psychiatry*, 78, 315-326.

Boissy, A., Arnould, C., Chaillou, E., Désiré, L., Duvaux-Ponter, C., Greiveldinger, L., Leterrier, C., Richard, S., Roussel, S., Saint-Dizier, H., Meunier-Salaün, MC., Valance, D., Veissier, I. (2007) Emotions and cognition: a new approach to animal welfare. *Animal Welfare*, 16, 37 – 43.

Bos, P.A., Hermans, E.J., Montoya, E.R., Ramsey, N.F. & van Honk, J., (2010) Testosterone administration modulates neural responses to crying infants in young females. *Psychoneuroendocrinology*, 35, 114-121

Boyle, L.A., Leonard, F.C., Lynch, P.B. & Brophy, P. (2002) Effect Of Gestation Housing On Behaviour And Skin Lesions Of Sows In Farrowing Crates. *Applied Animal Behaviour Science,* 76, 119 – 134.

Breier, B.H., Vickers, M.H., Ikenasio, B.A., Chan, K.Y. & Wong, W.P.S., (2001) Fetal programming of appetite and obesity. *Molecular and cellular endocrinology*, 185, 73-79.

Brouwers, E.P., van Baar, A.L. & Pop, V.J., (2001) Maternal anxiety during pregnancy and subsequent infant development. *Infant Behaviour and Development*, 24, .95-106.

Brydges, N.M., Leach, M., Nicol, K., Wright, R. & Bateson, M. (2011) Environmental enrichment induces optimistic cognitive bias in rats. *Animal Behaviour*, 81, 169-175.

Brydges, N.M., Hall, L., Nicolson, R., Holmes, M.C. & Hall, J., (2012) The effects of juvenile stress on anxiety, cognitive bias and decision making in adulthood: a rat model. *PLoS One*, 7, p.e48143.

Buckwalter, J.G., Stanczyk, F.Z., McCleary, C.A., Bluestein, B.W., Buckwalter, D.K., Rankin, K.P., Chang, L. & Goodwin, T.M. (1998) Pregnancy, The Postpartum, And Steroid Hormones: Effects On Cognition And Mood. *Psychoneuroendocrinology*, 24, 69 – 84.

Bunsey, M. & Eichenbaum, H. (1996) Conservation of hippocampal memory function in rats and humans. *Nature*, 379, 255.

Burgess, N., Maguire, E.A. & O'Keefe, J. (2002) The human hippocampus and spatial and episodic memory. *Neuron*, 35, 625-641.

Buss, C., Davis, E.P., Muftuler, L.T., Head, K. & Sandman, C.A., (2010) High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6–9-year-old children. *Psychoneuroendocrinology*, 35, 141-153.

Cameron, E.Z., Lemons, P.R., Bateman, P.W. & Bennett, N.C. (2008) Experimental alteration of litter sex ratios in a mammal. *Proceedings of the Royal Society of London B: Biological Sciences*, 275, 323-327.

Charil, A., Laplante, D.P., Vaillancourt, C. & King, S., (2010) Prenatal stress and brain development. *Brain research reviews*, 65, 56-79.

Christensen, H., Leach, L.S. and Mackinnon, A. (2010) Cognition in pregnancy and motherhood: prospective cohort study. *The British Journal of Psychiatry*, 196, 126-132.

Clark, M.M., Malenfant, S.A., Winter, D.A. & Galef, B.G. (1990) Fetal uterine position affects copulation and scent marking by adult male gerbils. *Physiology & behavior*, 47, 301-305.

Clark, M.M., Crews, D. & Galef, B.G. (1991) Concentrations of sex steroid hormones in pregnant and fetal Mongolian gerbils. *Physiology & behavior*, 49, 239-243.

Clark, M.M., Vom Saal, F.S. & Galef, B.G. (1992) Intrauterine positions and testosterone levels of adult male gerbils are correlated. *Physiology & behavior*, 51, 957-960.

Clarke, A.S., Wittwer, D.J., Abbott, D. & Schneider, M.L. (1994) Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. *Developmental psychobiology*, 27, 257-269.

Clark, M.M. & Galef, B.G. (1995) A gerbil dam's fetal intrauterine position affects the sex ratios of litters she gestates. *Physiology & behavior*, 57, 297-299.

Coe, C.L., Kramer, M., Czéh, B., Gould, E., Reeves, A.J., Kirschbaum, C. & Fuchs, E. (2003) Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biological psychiatry*, 54, 1025-1034.

Conte, S., Lawlor, P.G., O'Connell, N. & Boyle, L.A. (2012) Pigs
Effect Of Split Marketing On The Welfare, Performance, And Carcass Traits Of Finishing. *Journal Of Animal Science*, 90, 373 – 380.

Cost, K.T., Lobell, T.D., Williams-Yee, Z.N., Henderson, S. and Dohanich, G. (2014) The effects of pregnancy, lactation, and primiparity on object-in-place memory of female rats. *Hormones and behavior*, 65, 32-39.

Cottrell, E.C. & Seckl, J.R., (2009) Prenatal stress, glucocorticoids and the programming of adult disease. *Frontiers in behavioral neuroscience*, 3, 19.

Couret, D., Jamin, A., Kuntz-Simon, G., Prunier, A. & Merlot, E. (2009a) Maternal stress during late gestation has moderate but long-lasting effects on the immune system of the piglets. *Veterinary immunology and immunopathology*, 131, 17-24.

Couret, D., Prunier, A., Mounier, A.M., Thomas, F., Oswald, I.P. & Merlot, E. (2009b) Comparative effects of a prenatal stress occurring during early or late gestation on pig immune response. *Physiology & behavior*, 98, 498-504.

Crawley, R.A., Dennison, K. & Carter, C. (2003) Cognition in pregnancy and the first year post-partum. *Psychology and psychotherapy: Theory, research and practice*, 76, 69-84.

Curtis, S.E. & Stricklin, W.R., (1991) The importance of animal cognition in agricultural animal production systems: an overview. *Journal of animal science*, 69, 5001-5007.

Damm, B.I., Lisborg, L., Vestergaard, K.S. & Vanicek, J. (2003) Nest-Building, Behavioural Disturbances And Heart Rate In Farrowing Sows Kept In Crates And Schmid Pens. Livestock Science, 80, 175 – 187.

Darnaudéry, M., Perez-Martin, M., Del Favero, F., Gomez-Roldan, C., Garcia-Segura, L.M. & Maccari, S. (2007) Early motherhood in rats is associated with a modification of hippocampal function. *Psychoneuroendocrinology*, 32, 803-812.

Darnaudéry, M. & Maccari, S. (2008) Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain research reviews*, 57, 571-585.

Davis, E.P., Glynn, L.M., Waffarn, F. & Sandman, C.A. (2011) Prenatal maternal stress programs infant stress regulation. *Journal of Child Psychology and Psychiatry*, 52, 119-129.

D'Eath, R.B. & Lawrence, A.B. (2004) Early life predictors of the development of aggressive behaviour in the domestic pig. *Animal Behaviour*, *67,* 501-509.

D'Eath, R.B. (2005) Socialising piglets before weaning improves social hierarchy formation when pigs are mixed post-weaning. *Applied Animal Behaviour Science*, 93, 199-211.

De Groot, R.H.M., Vuurman, E.F.P.M., Hornstra, G. & Jolles, J. (2006) Differences in cognitive performance during pregnancy and early motherhood. *Psychological Medicine*, 36, 1023 – 1032.

del Mar Melero-Montes, M. & Jick, H., (2001) Hyperemesis gravidarum and the sex of the offspring. *Epidemiology*, 12, 123-124.

Dimitsantos, E., Escorihuela, R.M., Fuentes, S., Armario, A. & Nadal, R. (2007) Litter size affects emotionality in adult male rats. *Physiology & Behavior*, 92, 708-716.

Douglas, A.J., (2010) Baby love? Oxytocin-dopamine interactions in mother-infant bonding. *Endocrinology*, 151, 1978-1980.

Douglas, C., Bateson, M., Walsh, C., Bédué, A. and Edwards, S.A. (2012) Environmental Enrichment Induces Optimistic Cognitive Biases in Pigs. *Applied Animal Behaviour Science*, 139, 65-73.

Doyle, R.E., Vidal, S., Hinch, G.N., Fisher, A.D., Boissy, A. & Lee, C. (2010) The effect of repeated testing on judgement biases in sheep. *Behavioural Processes*, *83*, 349-352.

Drickamer, L.C., Arthur, R.D. & Rosenthal, T.L. (1997) Conception failure in swine: importance of the sex ratio of a female's birth litter and tests of other factors. *Journal of animal science*, 75, 2192-2196.

Duarte-Guterman, P., Yagi, S., Chow, C. & Galea, L.A. (2015) Hippocampal learning, memory, and neurogenesis: effects of sex and estrogens across the lifespan in adults. *Hormones and behavior*, 74, 37-52.

Duncan, I.J. & Petherick, J.C. (1991) The implications of cognitive processes for animal welfare. *Journal of animal science*, 69, 5017-5022.

Duncan, S. & Barrett, L.F. (2007) Affect is a form of cognition: A neurobiological analysis. *Cognition and emotion*, 21, 1184-1211.

Ericsson, K.A. & Kintsch, W. (1995) Long-term working memory. *Psychological review*, 102, 211.

Eriksson, J.G., Kajantie, E., Osmond, C., Thornburg, K. & Barker, D.J. (2010) Boys live dangerously in the womb. *American Journal of Human Biology*, 22, 330-335.

Evans, J., Heron, J., Francomb, H., Oke, S. & Golding, J. (2001) Cohort Study Of Depressed Mood During Pregnancy And After Childbirth. *British Medical Journal*, 323, 257 – 260.

Farrar, D., Tuffnell, D., Neill, J., Scally, A. & Marshall, K. (2014) Assessment of cognitive function across pregnancy using CANTAB: A longitudinal study. *Brain and Cognition*, 84, 76 – 84.

Fijn, L., Antonides, A., Aalderink, D., Nordquist, R.E. & van der Staay, F.J. (2016) Does litter size affect emotionality, spatial learning and memory in piglets? *Applied Animal Behaviour Science*, 178, 23-31.

Fisher, R.A. (1999) *The genetical theory of natural selection: a complete variorum edition*. Oxford University Press.

Francis, D.D., Young, L.J., Meaney, M.J. & Insel, T.R., (2002) Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. *Journal of neuroendocrinology*, 14, 349-353.

Friel, M., Kunc, H.P., Griffin, K., Asher, L. & Collins, L.M. (2016) Acoustic signalling reflects personality in a social mammal. *Open Science*, 3, 160178.

Frodl, T. & O'Keane, V. (2013) How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiology of disease*, 52, 24-37.

Food and agriculture organisation for the United Nations, (2017) Available from: http://www.fao.org/faostat/en/#home [Accessed on 13/09/2017]

Foxcroft, G.R., Dixon, W.T., Novak, S., Putman, C.T., Town, S.C. & Vinsky, M.D.A., (2006) The biological basis for prenatal programming of postnatal performance in pigs. *Journal of Animal Science*, 84, E105-E112.

Gabrieli, J.D., Fleischman, D.A., Keane, M.M., Reminger, S.L. & Morrell, F. (1995) Double dissociation between memory systems underlying explicit and implicit memory in the human brain. *Psychological Science*, 6, 76-82.

Gaillard, A., Le Strat, Y., Mandelbrot, L., Keïta, H. & Dubertret, C. (2014) Predictors of postpartum depression: Prospective study of 264 women followed during pregnancy and postpartum. *Psychiatry research*, *215*, 341-346.

Galea, L.A., Ormerod, B.K., Sampath, S., Kostaras, X., Wilkie, D.M. & Phelps, M.T., (2000) Spatial working memory and hippocampal size across pregnancy in rats. *Hormones and behavior*, 37,.86-95.

Galea, L.A., Uban, K.A., Epp, J.R., Brummelte, S., Barha, C.K., Wilson, W.L., Lieblich, S.E. & Pawluski, J.L. (2008) Endocrine regulation of cognition and neuroplasticity: our pursuit to unveil the complex interaction between hormones, the brain, and behaviour. *Canadian Journal of Experimental Psychology/Revue canadienne de psychologie expérimentale*, 62, 247.

Galea, L.A.M., Wainwright, S.R., Roes, M.M., Duarte-Guterman, P., Chow, C. & Hamson, D.K. (2013) Sex, hormones and neurogenesis in the hippocampus: hormonal modulation of neurogenesis and potential functional implications. *Journal of neuroendocrinology*, 25, 1039-1061.

Gicquel, C., El-Osta, A. & Le Bouc, Y. (2008) Epigenetic regulation and fetal programming. *Best practice & research Clinical endocrinology & metabolism*, 22, 1-16.

Gieling, E.T., Nordquist, R.E. & van der Staay, F.J. (2011a) Assessing learning and memory in pigs. *Animal Cognition*, 14, 151 – 173.

Gieling, E.T., Schuurman, T., Nordquist, R.E. & van der Staay, F.J. (2011b) The pig as a model animal for studying cognition and neurobehavioral disorders. *Current Topics In Behavioural Neurosciences.* 7, 359 – 383.

Gieling, E.T., Park, S.Y., Nordquist, R.E. & van der Staay, F.J. (2012) Cognitive performance of low-and normal-birth-weight piglets in a spatial hole-board discrimination task. *Pediatric research*, 71, 71-76.

Grandinson, K. (2005) Genetic background of maternal behaviour and its relation to offspring survival. *Livestock Production Science*, 93, 43-50.

Grant, V.J. (2007) Could maternal testosterone levels govern mammalian sex ratio deviations? *Journal of Theoretical Biology*, 246, 708 – 719.

Griffin, A.S., Guillette, L.M. & Healy, S.D. (2015) Cognition and personality: an analysis of an emerging field. *Trends in ecology & evolution*, 30, 207-214.

Glover, V., O'connor, T.G. & O'Donnell, K. (2010) Prenatal stress and the programming of the HPA axis. *Neuroscience & Biobehavioral Reviews*, 35, 17-22.

Gluckman, P.D., Hanson, M.A., Cooper, C. & Thornburg, K.L. (2008) Effect of in utero and early-life conditions on adult health and disease. *New England Journal of Medicine*, 359, 61-73.

Glynn, L.M. (2010) Giving birth to a new brain: hormone exposures of pregnancy influence human memory. *Psychoneuroendocrinology*, 35, 1148-1155.

Godfrey, K.M. & Barker, D.J., (2000) Fetal nutrition and adult disease. *The American journal of clinical nutrition*, 71, 1344s-1352s.

González-Menéndez, I., Contreras, F., García-Fernández, J.M. & Cernuda-Cernuda, R., (2011) Perinatal development of melanopsin expression in the mouse retina. *Brain research*, 1419, 12-18.

Gorecki, M.T. (2003) Sex ratio in litters of domestic pigs (Sus scrofa f. domestica Linnaeus, 1758). *Biological Letters*, 40, 111-118.

Gorissen, B., Wolschrijn, C.F., van Vilsteren, A.A., van Rietbergen, B. & van Weeren, P.R. (2016) Trabecular bone of precocials at birth; Are they prepared to run for the wolf (f)? *Journal of morphology*, 277, 948-956.

Hampson, E., Phillips, S.D., Duff-Canning, S.J., Evans, K.L., Merrill, M., Pinsonneault, J.K., Sadée, W., Soares, C.N. & Steiner, M. (2015) Working memory in pregnant women: relation to estrogen and antepartum depression. *Hormones and behavior*, *74*, 218-227.

Hanson, M.A. & Gluckman, P.D. (2008) Developmental origins of health and disease: new insights. *Basic & clinical pharmacology & toxicology*, 102, 90-93.

Harris, A. & Seckl, J. (2011) Glucocorticoids, prenatal stress and the programming of disease. *Hormones and behavior*, 59, 279-289.

Heerwagen, M.J., Miller, M.R., Barbour, L.A. & Friedman, J.E., (2010) Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299, 711-R722.

Held, S., Mendl, M., Laughlin, K. and Byrne, R.W., (2002) Cognition studies with pigs: livestock cognition and its implication for production. *Journal of Animal Science*, 80, E10-E17.

Henry, C., Kabbaj, M., Simon, H., Moal, M. & Maccari, S. (1994) Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *Journal of neuroendocrinology*, 6, 341-345.

Henry, J.D. & Rendell, P.G. (2007) A review of the impact of pregnancy on memory function. *Journal of Clinical and Experimental Neuropsychology*, 29, 793-803.

Herpin, P., Damon, M. and Le Dividich, J. (2002) Development of thermoregulation and neonatal survival in pigs. *Livestock production science*, 78, 25-45.

Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J.C., Tobeña, A., Desco, M., Crone, E.A. & Ballesteros, A. (2017). Pregnancy leads to long-lasting changes in human brain structure. *Nature Neuroscience*, 20, 287-296.

Huang, J.S., Lee, T.A. & Lu, M.C., (2007) Prenatal programming of childhood overweight and obesity. *Maternal and child health journal*, 11, 461-473.

Jacobson, L. & Sapolsky, R. (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine reviews*, 12, 118-134.

Jarvis, S., Calvert, S.K., Stevenson, J., VanLeeuwen, N. & Lawrence, A.B. (2002) Pituitary-adrenal activation in pre-parturient pigs (Sus scrofa) is associated with behavioural restriction due to lack of space rather than nesting substrate. *Animal Welfare*, 11, 371-384.

Jarvis, S., Moinard, C., Robson, S.K., Baxter, E., Ormandy, E., Douglas, A.J., Seckl, J.R., Russell, J.A. & Lawrence, A.B. (2006) Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. *Hormones and Behavior*, 49, 68-80.

Jelsing, J., Nielsen, R., Olsen, A.K., Grand, N., Hemmingsen, R. & Pakkenberg, B. (2006) The postnatal development of neocortical neurons and glial cells in the Göttingen minipig and the domestic pig brain. *Journal of Experimental Biology*, 209, 1454-1462.

Keenan, P.A., Yaldoo, D.T., Fuerst, D.R. & Ginsburg, K.A. (1998) Explicit memory in pregnant women. *American journal of obstetrics and gynecology*, 179, 731-737.

Kendrick, K.M., Keverne, E.B. & Baldwin, B.A., (1987) Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. *Neuroendocrinology*, 46, 56-61.

Keyte, A.L. & Smith, K.K. (2010) Developmental origins of precocial forelimbs in marsupial neonates. *Development*, 137, 4283-4294.

Kim, P., Strathearn, L. & Swain, J.E., (2016) The maternal brain and its plasticity in humans. *Hormones and Behavior*, 77, 113-123.

Kinsley, C.H., Madonia, L., Gifford, G.W., Tureski, K., Griffin, G.R., Lowry, C., Williams, J., Collins, J., McLearie, H. & Lambert, K.G., (1999) Motherhood improves learning and memory. *Nature*, 402, 137.

Kinsley, C.H., Trainer, R., Stafisso-Sandoz, G., Quadros, P., Marcus, L.K., Hearon, C., Meyer, E.A.A., Hester, N., Morgan, M., Kozub, F.J. & Lambert, K.G. (2006) Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. *Hormones and behavior*, 49, 131-142.

Kofman, O. (2002) The role of prenatal stress in the etiology of developmental behavioural disorders. *Neuroscience & Biobehavioral Reviews*, 26, .457-470.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W. and Blokhuis, H.J. (1999) Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, 23, 925-935.

Kornum, B.R. & Knudsen, G.M. (2011) Cognitive testing of pigs (*Sus scrofa*) in translational biobehavioral research. *Neuroscience and Biobehavioural Reviews*. 35, 437 – 451.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2016) lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest

Lamireau, D., Nuyt, A.M., Hou, X., Bernier, S., Beauchamp, M., Gobeil, F., Lahaie, I., Varma, D.R. & Chemtob, S. (2002) Altered vascular function in fetal programming of hypertension. *Stroke*, 33, 2992-2998.

Lancaster, C.A., Gold, K.J., Flynn, H.A., Yoo, H., Marcus, S.M. & Davis, M.M. (2010) Risk factors for depressive symptoms during pregnancy: a systematic review. *American journal of obstetrics and gynecology*, 202, 5-14.

Leiser, R. & Kaufmann, P. (1994) Placental structure: in a comparative aspect. *Experimental and Clinical Endocrinology & Diabetes*, 102, 122-134.

Lemaire, V., Koehl, M., Le Moal, M. & Abrous, D.N. (2000) Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences*, 97, 11032-11037.

Leon, J.B., Smith, B.B., Timm, K.I. & LeCren, G., (1990) Endocrine changes during pregnancy, parturition and the early post-partum period in the llama (Lama glama). *Journal of reproduction and fertility*, 88, 503-511.

Leung, S., Arthur, D.G. & Martinson, I., (2005) Stress in women with postpartum depression: a phenomenological study. *Journal of Advanced Nursing*, 51, 353-360.

Lind, N.M., Moustgaard, A., Jelsing, J., Vajta, G., Cumming, P. & Hansen, A.K, (2007) The use of pigs in neuroscience: modeling brain disorders. *Neuroscience & Biobehavioral Reviews*, 31, 728-751.

Liu, D., Diorio, J., Day, J.C., Francis, D.D. & Meaney, M.J., (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature neuroscience*, 3, 799.

Lodge, C.J., Allen, K.J., Lowe, A.J., Hill, D.J., Hosking, C.S., Abramson, M.J. & Dharmage, S.C. (2011) Perinatal cat and dog exposure and the risk of asthma and allergy in the urban environment: a systematic review of longitudinal studies. *Clinical and Developmental Immunology*, 2012

Logan, D.M., Hill, K.R., Jones, R., Holt-Lunstad, J. & Larson, M.J. (2014) How do memory and attention change with pregnancy and childbirth? A controlled longitudinal examination of neuropsychological functioning in pregnant and postpartum women. *Journal of clinical and experimental neuropsychology*, 36, 528-539.

Lombardo, M.V., Ashwin, E., Auyeung, B., Chakrabarti, B., Taylor, K., Hackett, G., Bullmore, E.T. & Baron-Cohen, S., (2012) Fetal testosterone influences sexually dimorphic gray matter in the human brain. *Journal of Neuroscience*, 32, 674-680.

Lothmann, C., Holmes, E.A., Chan, S.W.Y. & Lau, J.Y.F. (2010) Cognitive bias modification training in adolescents: effects on interpretation biases and mood. *The Journal Of Child Psychology And Psychiatry*, 52, 24 – 32.

Love, O.P. & Williams, T.D. (2008) Plasticity in the adrenocortical response of a free-living vertebrate: the role of pre-and post-natal developmental stress. *Hormones and Behavior*, 54, 496-505.

Lovejoy, M.C., Graczyk, P.A., O'Hare, E. & Neuman, G. (2000) Maternal depression and parenting behavior: A meta-analytic review. *Clinical psychology review*, 20, 561-592.

Lui, C.C., Wang, J.Y., Tain, Y.L., Chen, Y.C., Chang, K.A., Lai, M.C. & Huang, L.T. (2011) Prenatal stress in rat causes long-term spatial memory deficit and hippocampus MRI abnormality: Differential effects of postweaning enriched environment. *Neurochemistry international*, 58, 434-441.

Macbeth, A.H. & Luine, V.N. (2010) Changes in anxiety and cognition due to reproductive experience: a review of data from rodent and human mothers. *Neuroscience & Biobehavioral Reviews*, 34, 452-467.

Marchant-Forde, J.N. & Marchant-Forde, R.M. (2005) Minimizing inter-pig aggression during mixing. *Pig News and Information*, 26, 63N.

Marcus, S.M., Flynn, H.A., Blow, F.C. & Barry, K.L. (2003) Depressive symptoms among pregnant women screened in obstetrics settings. *Journal of Women's Health*, 12, 373-380.

Marino, S.E., Meador, K.J., Loring, D.W., Okun, M.S., Fernandez, H.H., Fessler, A.J, Kustra, R.P., Miller, J.M., Ray. P.J., Roy, A., Schoenberg, M.R., Vahle, V.J. & Werz, M.A. (2009) Subjective perception of cognition is related to mood and not performance. *Epilepsy Behaviour*, 14, 459 – 464.

Martin, J.E., Ison, S.H. & Baxter, E.M. (2015) The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. *Applied Animal Behaviour Science*, 163, 69-79.

Mate, K., Robinson, E.S., A Pedersen, R. & L Vandeberg, J., (1994) Timetable of in vivo embryonic development in the grey short-tailed opossum (Monodelphis domestica). *Molecular reproduction and development*, 29, 365-374.

Mathews, A. & MacLeod, C. (2005) Cognitive vulnerability to emotional disorders. *Annual Review of Clinical Psychology*, 1, 167-195.

McFadden, D. (1993) A masculinizing effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences*, 90, 11900-11904.

Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E. and Casey, R. (2010) Dogs Showing Separation-Related Behaviour Exhibit A 'Pessimistic' Cognitive Bias. *Current Biology*, 20, 839-840.

Merlot, E., Couret, D. & Otten, W. (2008) Prenatal stress, fetal imprinting and immunity. *Brain, behavior, and immunity*, *22*, 42-51.

Merlot, E., Quesnel, H. & Prunier, A. (2015) Consequences of maternal stress during gestation on immunity and health of new-born animals in farm species. *INRA PRODUCTIONS ANIMALES*, *28*, 283-293.

Merrifield, C.A., Lewis, M., Claus, S.P., Beckonert, O.P., Dumas, M.E., Duncker, S., Kochhar, S., Rezzi, S., Lindon, J.C., Bailey, M. & Holmes, E. (2011) A metabolic system-wide characterisation of the pig: a model for human physiology. *Molecular BioSystems*, 7, 2577-2588.

Milligan, B.N., Fraser, D. & Kramer, D.L. (2002) Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. *Livestock Production Science*, 76, 181-191.

Mueller, B.R. & Bale, T.L., (2008) Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience*, 28, 9055-9065.

Murray, C.M., Stanton, M.A., Wellens, K.R., Santymire, R.M., Heintz, M.R. & Lonsdorf, E.V., (2016) Maternal effects on offspring stress physiology in wild chimpanzees. *American journal of primatology*. 10.1002/ajp.22525.

Nettle, D., Monaghan, P., Boner, W., Gillespie, R. & Bateson, M. (2013) Bottom of the heap: having heavier competitors accelerates early-life telomere loss in the European starling, Sturnus vulgaris. *PLoS One*, 8, p.e83617.

Nissen, E., Lilja, G., Widström, A.M. & Uvnás-Moberg, K., (1995) Elevation of oxytocin levels early post partum in women. *Acta obstetricia et gynecologica Scandinavica*, 74, 530-533.

Nyman, A.J. (1967) Problem solving in rats as a function of experience at different ages. *The Journal of genetic psychology*, 110, 31-39.

Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J.V., Puri, B.K., Fusi, L. & Bydder, G.M. (2002) Change in brain size during and after pregnancy: study in healthy women and women with preeclampsia. *American Journal of Neuroradiology*, 23, 19-26.

Oliviero, C., Heinonen, M., Valros, A., Hälli, O. & Peltoniemi, O.A.T. (2008) Effect of the environment on the physiology of the sow during late pregnancy, farrowing and early lactation. *Animal reproduction science*, *105*, 365-377.

Otten, W., Kanitz, E., Couret, D., Veissier, I., Prunier, A. & Merlot, E. (2010) Maternal social stress during late pregnancy affects hypothalamic-pituitary-adrenal function and brain neurotransmitter systems in pig offspring. *Domestic animal endocrinology*, 38, 146-156.

Pawluski, J.L., Walker, S.K. & Galea, L.A. (2006) Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Hormones and behavior*, 49, 143-149.

Pawluski, J.L., Lambert, K.G. & Kinsley, C.H. (2016) Neuroplasticity in the maternal hippocampus: relation to cognition and effects of repeated stress. *Hormones and Behavior*, 77, 86-97.

Peaker, M. & Taylor, E. (1996) Sex ratio and litter size in the guinea-pig. *Journal of reproduction and fertility*, 108, 63-67.

Pond, W.G., Boleman, S.L., Fiorotto, M.L., Ho, H., Knabe, D.A., Mersmann, H.J., Savell, J.W. & Su, D.R. (2000) Perinatal ontogeny of brain growth in the domestic pig. *Proceedings of the Society for Experimental Biology and Medicine*, 223, 102-108.

Purl, C.P. & Garfield, R.E., (1982) Changes in hormone levels and gap junctions in the rat uterus during pregnancy and parturition. *Biology of reproduction*, 27, 967-975.

Quiniou, N., Dagorn, J. & Gaudré, D. (2002) Variation of piglets' birth weight and consequences on subsequent performance. *Livestock Production Science*, 78, 63-70.

Rich-Edwards, J.W., Kleinman, K., Abrams, A., Harlow, B.L., McLaughlin, T.J., Joffe, H. & Gillman, M.W. (2006) Sociodemographic predictors of antenatal and postpartum depressive symptoms among women in a medical group practice. *Journal of Epidemiology & Community Health*, 60, 221-227.

Riem, M.M., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., Out, D. & Rombouts, S.A., (2012) Attachment in the brain: adult attachment representations predict amygdala and behavioral responses to infant crying. *Attachment & human development*, 14, 533-551.

Robertson, H.A. & King, G.J., (1974) Plasma Concentrations Of Progesterone, Oestrone, Oestrodial-17 β And Of Oestrone Sulphate In The Pig At Implantation, During Pregnancy and Parturition. *The Journal Of The Society For Reproduction And Fertility*, 40, 133 – 141

Rooke, J.A., Arnott, G., Dwyer, C.M. & Rutherford, K.M.D. (2015) The importance of the gestation period for welfare of lambs: maternal stressors and lamb vigour and wellbeing. *The Journal of Agricultural Science*, *153*, 497-519.

Rooke, J.A., Arnott, G., Dwyer, C.M. & Rutherford, K.M.D. (2017) Impact of maternal stress and nutrition on behavioural and physiological outcomes in young lambs. *Animal Welfare*, *26*(4), 403-415.

Rosenberg, K. & Trevathan, W. (2002) Birth, obstetrics and human evolution. *BJOG: An International Journal of Obstetrics & Gynaecology*, 109, 1199-1206.

Ross, H.E. and Young, L.J., (2009) Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Frontiers in neuroendocrinology*, 30, 534-547.

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL: http://www.rstudio.com/

Rutkowska, J. & Cichoń, M., (2006) Maternal testosterone affects the primary sex ratio and offspring survival in zebra finches. *Animal Behaviour*, 71, 1283-1288.

Rutherford, K.M.D., Baxter, E.M., D'Eath, R.B., Turner, S.P., Arnott, G., Roehe, R., Ask, B., Sandøe, P., Moustsen, V.A., Thorup, F. & Edwards, S.A. (2013) The welfare implications of large litter size in the domestic pig I: biological factors. *Animal Welfare*, 22, 199-218.

Rutherford, K.M., Piastowska-Ciesielska, A., Donald, R.D., Robson, S.K., Ison, S.H., Jarvis, S., Brunton, P.J., Russell, J.A. & Lawrence, A.B. (2014) Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig. *Physiology & behavior*, *129*, 255-264.

Ryan, B.C. & Vandenbergh, J.G. (2002) Intrauterine position effects. *Neuroscience & Biobehavioral Reviews*, 26, 665-678.

Ryan, S.J., Knechtel, C.U. & Getz, W.M., (2007). Ecological cues, gestation length, and birth timing in African buffalo (Syncerus caffer). *Behavioral Ecology*, 18, 635-644.

Sandman, C.A., Glynn, L.M. & Davis, E.P. (2013) Is there a viability–vulnerability tradeoff? Sex differences in fetal programming. *Journal of psychosomatic research*, 75, 327-335.

Schrøder-Petersen, D.L. & Simonsen, H.B. (2001) Tail biting in pigs. *The Veterinary Journal*, 162, 196-210.

Scollo, A., Gottardo, F., Contiero, B. and Edwards, S.A. (2014) Does Stocking Density Modify Affective State In Pigs As Assessed By Cognitive Bias, Behavioral And Physiological Parameters? *Applied Animal Behaviour Science*, 153, 26-35.

Schwarzenberger, F., Fredriksson, G., Schaller, K. & Kolter, L., (2004) Fecal steroid analysis for monitoring reproduction in the sun bear (Helarctos malayanus). *Theriogenology*, 62, 1677-1692.

Seckl, J.R. & Holmes, M.C. (2007) Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nature Reviews. Endocrinology*, 3, 479.

Servanty, S., Gaillard, J.M., Allainé, D., Brandt, S. & Baubet, E. (2007) Litter size and fetal sex ratio adjustment in a highly polytocous species: the wild boar. *Behavioral Ecology*, 18, 427-432.

Sharot, T. (2011) The optimism bias. *Current biology*, 21, 941-945.

Sherry, D.F., Galef Jr, B.G. & Clark, M.M. (1996) Sex and intrauterine position influence the size of the gerbil hippocampus. *Physiology & behavior*, *60*, 1491-1494.

Sih, A., Bell, A.M., Johnson, J.C. and Ziemba, R.E. (2004) Behavioral syndromes: an integrative overview. *The quarterly review of biology*, 79.241-277.

Silk, J., Short, J., Roberts, J. & Kusnitz, J. (1993) Gestation length in rhesus macaques (Macaca mulatta). *International Journal of Primatology*, 14, 95-104.

Soldin, O.P., Guo, T., Weiderpass, E., Tractenberg, R.E., Hilakivi-Clarke, L. & Soldin, S.J. (2005) Steroid Hormone Levels In Pregnancy And 1 Year Postpartum Using Isotope Dilution Tandem Mass Spectrometry. *Fertility And Sterility*, 84, 701 – 710.

Sousa, V.C., Vital, J., Costenla, A.R., Batalha, V.L., Sebastião, A.M., Ribeiro, J.A. & Lopes, L.V., (2014) Maternal separation impairs long term-potentiation in CA1-CA3 synapses and hippocampal-dependent memory in old rats. *Neurobiology of aging*, 35, 1680-1685.

Stevens, K.N., Asher, L., Griffin, K., Friel, M., O'Connell, N. & Collins, L.M. (2017) A comparison of inferential analysis methods for multilevel studies: Implications for drawing conclusions in animal welfare science. *Applied Animal Behaviour Science*, https://doi.org/10.1016/j.applanim.2017.08.002

Strathearn, L., Li, J., Fonagy, P. & Montague, P.R. (2008) What's in a smile? Maternal brain responses to infant facial cues. *Pediatrics*, 122, 40-51.

Stukenborg, A., Traulsen, I., Puppe, B., Presuhn, U. & Krieter, J. (2011) Agonistic behaviour after mixing in pigs under commercial farm conditions. *Applied Animal Behaviour Science*, 129, 28-35.

Steiner, M., Dunn, E. & Born, L (2003) Hormones And Mood: From Menarche To Menopause And Beyond. *Journal of Affective Disorder*, 74, 67 – 83.

Strunk, D.R., Lopez, H. & DeRubeis, R.J. (2006) Depressive symptoms are associated with unrealistic negative predictions of future life events. *Behaviour research and therapy*, 44, 861-882.

Sullivan, T.P., Eaglstein, W.H., Davis, S.C. & Mertz, P. (2001) The pig as a model for human wound healing. *Wound repair and regeneration*, 9, 66-76.

Sullivan, R.M. (2004) Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. *Stress*, 7, 131-143.

Sun, T. & Hevner, R.F. (2014) Growth and folding of the mammalian cerebral cortex: from molecules to malformations. *Nature Reviews Neuroscience*, 15, 217-232.

Sutherland, M.A. & Tucker, C.B. (2011) The long and short of it: A review of tail docking in farm animals. *Applied Animal Behaviour Science*, 135, 179-191.

Szuran, T., Zimmermann, E. & Welzl, H. (1994) Water maze performance and hippocampal weight of prenatally stressed rats. *Behavioural brain research*, 65, 153-155.

Tamaroff, M.H., Nir, Y. & Straker, N., (1986) Children reared in a reverse isolation environment: Effects on cognitive and emotional development. *Journal of autism and developmental disorders*, 16, 415-424.

Tang, A.C. & Verstynen, T. (2002) Early life environment modulates 'handedness' in rats. *Behavioural brain research*, 131, 1-7.

Tesfu, F., Gebrekidan, B. & Afera, B. (2014) Assessment and comparison of sex ratio following artificial insemination and natural mating in small scale and Modern dairy cattle farms in Mekelle. *Journal of Reproduction and Infertility*, 5, 58 – 64.

Teixeira, C., Figueiredo, B., Conde, A., Pacheco, A. & Costa, R. (2009) Anxiety and depression during pregnancy in women and men. *Journal of affective disorders*, 119, 142-148.

Thodberg, K., Jensen, K.H. & Herskin, M.S. (2002) Nest building and farrowing in sows: relation to the reaction pattern during stress, farrowing environment and experience. *Applied Animal Behaviour Science*, 77, 21-42.

Trivers, R.L. & Willard, D.E. (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science*, 179, 90-92.

Tuchscherer, M., Kanitz, E., Otten, W. & Tuchscherer, A. (2002) Effects of prenatal stress on cellular and humoral immune responses in neonatal pigs. *Veterinary immunology and immunopathology*, 86, 195-203.

Turner, S.P., Farnworth, M.J., White, I.M., Brotherstone, S., Mendl, M., Knap, P., Penny, P. & Lawrence, A.B. (2006) The accumulation of skin lesions and their use as a predictor of individual aggressiveness in pigs. *Applied Animal Behaviour Science*, 96, 245-259.

Uguz, F., Gezginc, K., Kayhan, F., Sari, S. & Buyukoz, D. (2010) Is Pregnancy Associated With Mood And Anxiety Disorders? A Cross-Sectional Study. *General Hospital Psychiatry*, 32, 213 – 215.

Vandenbergh, J.G. & Huggett, C.L. (1994) Mother's prior intrauterine position affects the sex ratio of her offspring in house mice. *Proceedings of the National Academy of Sciences*, 91, 11055-11059.

Van den Bergh, B.R., Mennes, M., Oosterlaan, J., Stevens, V., Stiers, P., Marcoen, A. & Lagae, L., (2005) High antenatal maternal anxiety is related to impulsivity during performance on cognitive tasks in 14-and 15-year-olds. *Neuroscience & Biobehavioral Reviews*, 29, 259-269.

Van den Bergh, B.R., Van Calster, B., Smits, T., Van Huffel, S. & Lagae, L. (2008) Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology*, 33, 536.

Vanston, C.M. & Watson, N.V., (2005) Selective and persistent effect of foetal sex on cognition in pregnant women. *Neuroreport*, 16, 779-782.

Vatten, L.J. & Skjærven, R., (2004) Offspring sex and pregnancy outcome by length of gestation. *Early human development*, 76, 47-54.

Vehaskari, V.M., Aviles, D.H. & Manning, J., (2001) Prenatal programming of adult hypertension in the rat. *Kidney international*, 59, 238-245.

Veiga, J.P., Viñuela, J., Cordero, P.J., Aparicio, J.M. & Polo, V., (2004) Experimentally increased testosterone affects social rank and primary sex ratio in the spotless starling. *Hormones and Behavior*, 46, 47-53.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

Warner, M.J. & Ozanne, S.E. (2010) Mechanisms involved in the developmental programming of adulthood disease. *Biochemical Journal*, 427, 333-347.

Weary, D.M., Pajor, E.A., Fraser, D. & Honkanen, A.M. (1996) Sow body movements that crush piglets: a comparison between two types of farrowing accommodation. *Applied Animal Behaviour Science*, 49, 149-158.

Weber, R., Keil, N.M., Fehr, M. & Horat, R. (2007) Piglet mortality on farms using farrowing systems with or without crates. *Animal Welfare*, 16, 277-279.

Wilsson, E. & Sundgren, P.E. (1998) Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. *Applied Animal Behaviour Science*, 56, 245-254.

Wischner, D., Kemper, N. & Krieter, J. (2009) Nest-Building Behaviour In Sows And Concequences For Pig Husbandry. *Livestock Science*, 124, 1 – 8.

Workman, J.L., Barha, C.K. and Galea, L.A.M. (2011) Endocrine Substrates Of Cognitive And Affective Changes During Pregnancy And Postpartum. *Behavioural Neuroscience*, 126, 54-72.

World Health Organisation (2017), Maternal and perinatal health, Available from: http://www.who.int/maternal_child_adolescent/topics/maternal/maternal_perinatal/en/ [Accessed on 13/09/2017]

Yang, J., Han, H., Cao, J., Li, L. & Xu, L. (2006) Prenatal stress modifies hippocampal synaptic plasticity and spatial learning in young rat offspring. *Hippocampus*, 16, 431-436.

Yang, J., Hou, C., Ma, N., Liu, J., Zhang, Y., Zhou, J., Xu, L. & Li, L. (2007) Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive

deficits induced by prenatal chronic stress. *Neurobiology of learning and memory*, 87, 257-263.

Zuena, A.R., Mairesse, J., Casolini, P., Cinque, C., Alemà, G.S., Morley-Fletcher, S., Chiodi, V., Spagnoli, L.G., Gradini, R., Catalani, A. & Nicoletti, F. (2008) Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. *PloS one*, 3, 2170.